THE EFFECT OF QOI FUNGICIDES ON MONOCYCLIC COMPONENTS OF PEACH BROWN ROT EPIDEMICS CAUSED BY *MONILINIA FRUCTICOLA*

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ABSTRACT OF THE THESIS

THE EFFECT OF QOI FUNGICIDES ON MONOCYCLIC COMPONENTS OF PEACH BROWN ROT EPIDEMICS CAUSED BY MONILINIA FRUCTICOLA

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Brown rot is the most significant disease infecting peach orchards in the United States and is caused by the ascomycete *Monilinia fructicola*. Blossom blight, twig cankers and fruit rot are disease symptoms that develop over the course of a season. Demethylation inhibitor fungicides, or DMIs, have been used for over twenty years to effectively control brown rot. Quinone outside inhibitors (QoIs), also known as strobilurin fungicides, are a relatively new class of fungicides previously shown to control brown rot when applied as protectants. We examined the effects of azoxystrobin, trifloxystrobin and a mixture of pyraclostrobin + boscalid on blossom blight and fruit infections. Peach trees were sprayed at different rates, volumes and timing intervals to investigate the possible curative properties of these strobilurins. Results showed that the most effective fungicide to control colonization of a peach fruit was azoxystrobin. Sporulation of fruit infections, as well as blossom blight cankers, was best controlled by applications of trifloxystrobin. This fungicide reduced sporulation by 89% on fruit and

71% on cankers when applied at the highest labeled rate. These data indicated that in addition to their known protectant activity, QoI fungicides exhibit specific curative properties that provide control during other phases of the brown rot infection cycle. In recent years in the southeastern United States peach growing region, studies have found DMI-resistant isolates of *M. fructicola*. Eleven isolates of *M. fructicola* taken from Southern New Jersey were screened for resistance using a PCR-RFLP method. One out of the eleven isolates examined showed similar genetic components to those isolates resistant to DMI fungicides, indicating that resistant strains exist in New Jersey orchards. Given this finding, the incorporation of strobilurins into the commercial spray program will be an important and necessary strategy to avoiding widespread DMI resistance in New Jersey. Since our results demonstrated good to excellent curative properties, particularly as anti-sporulants, we hypothesize that early- to mid-season deployment of the strobilurins will provide the greatest benefit in reducing development of brown rot epidemics during the harvest season.

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1. LITERATURE REVIEW

Brown rot is a devastating disease to peach orchards worldwide. Brown rot also affects other stone and pome fruit trees such as plum, apricot, cherry, apple and pear. The United States, a major producer of stone fruits, is at risk for potentially large yield losses each year due to brown rot. According to the United States Department of Agriculture, in 2006 the United States produced 916,347 tons of peaches; 268,218 tons of sweet cherries; 44,480 tons of apricots and 19,504 tons of prunes and plums. New Jersey is the fourth highest peach producer in the country next to California, Georgia and South Carolina. In 2005, New Jersey produced 35,000 tons of peaches over 7,400 acres of land. Peaches are economically important, second only to blueberries, as the most valuable crop bringing in 30.9 million dollars (Joshua, 2006).

1.1 The Pathogen

The causal agent of brown rot on peach found in North and South America, Australia and Southern Asia is *Monilinia fructicola* (G. Wint. Honey) (Anderson, 1956). Closely related pathogens, *M. fructigena* and *M. laxa* cause brown rot in Europe and Western United States. The fungal pathogen *M. fructicola* is an ascomycete that is part of the class Leotiomycetes and the order Helotiales, which is the largest order of inoperculate discomycetes (Alexopoulos *et al*., 1996). *Monilinia* is placed in the Sclerotiniaceae family and closely related to *Sclerotinia*. The two genera are so similar, when originally described in the late 1800's they were both placed under the *Sclerotinia* genus. It was not until 1928 when Honey proposed that fungi with moniloid conidia and pseudosclerotia be placed into a new genus, *Monilinia* (Anderson, 1956). Despite the acceptance of this proposal, the old name of *Sclerotinia* was still commonly used

throughout the literature up until the 1950's (Anderson, 1956). The official name of the fungus is *Monilinia fructicola* described by (G. Wint) Honey (Anderson, 1956).

Monilinia fructicola has a sexual and asexual phase. The sexual phase is very rarely known to occur in the Eastern United States. The fruiting body of the perfect stage is an apothecium, which is shortly stalked and cupulate like and arises most commonly from stromata or scleroita found in mummies that have fallen to the ground (Anderson, 1956; Whetzel, 1945; Alexopoulos *et al*. 1996). The apothecia then form asci that bear eight single-celled elliptical ascospores (Anderson, 1956). The sexual phase occurs as the weather warms in the spring and new shoots begin to emerge and flowers begin to blossom.

The asexual phase is largely responsible for causing brown rot on fruits in the United States. This imperfect state consists mostly of mycelia and conidia. The mycelium is described as hyphae that are clear, septate and branched (Anderson, 1956). Conidiophores are specialized hypae, typically long and branched, which bear chains of single celled oval or lemon shaped conidia (Alexopoulos *et al*. 1996; Whetzel, 1945). Conidia are easily dispersed by wind and rain and will form appressoria or begin to germinate which cause infection to blossoms, twigs and fruit (Alexopoulos *et al.* 1996).

1.2 The Disease Cycle

Over the course of the polycyclic disease cycle of brown rot, there are three major symptoms commonly associated with *M. fructicola* infection responsible for yield loss. In the spring, particularly between bud break and soon after petal fall, overwintering mummies produce lots of conidia that infect many blossoms. The infected blossoms develop brown lesions on the stamens, pistils and petals and in only three days after

infection can become shriveled and blighted. Blossom blight can occur from 10°C to 20°C along with a period of at least 80% humidity for close to 10 hours (Weaver, 1950). This symptom is not a major cause for yield loss even though no fruit can be produced by a blighted blossom. Peach trees produce enormous amounts of flowers and since the incidence of blossom blight is generally low, there is little or no fruit loss.

After a blossom has been infected by *M. fructicola*, the pathogen can travel into the connecting twig resulting in the formation of a twig canker. These twig cankers can cause an infected area of the twig around the blighted blossom to become gummy. They may grow large enough to girdle the twig and cause severe damage to the twig and the subsequent developing fruit attached. Fruit infection from spores produced on twig cankers and blighted blossoms has the greatest impact on crop yield reduction. Temperatures required for sporulation are between 15°C and 25°C with ideal conditions around 20°C (Phillips, 1984). A few studies have also shown the success of the pathogen overwintering as mycelia in twig cankers (Byrde *et al.*, 1977).

Fruit rot most commonly develops at the ripening and maturing stages of peach development (Biggs and Northover, 1988; Emery *et al*. 2000). As fruit begins to ripen, the increase in water forces the epidermal cells to expand which may serve as a weak point of entry for the fungus (Corbin, 1962). Fruit are most susceptible to infection during this period of development. Although fruit rot can occur on immature fruit, its occurrence is rare and usually related to penetration via stomata, environmental injury, or insect wounds. Latent and quiescent infections can occur on immature fruit by conidia forming appressoria to penetrate the cuticle layers (Lee and Bostock, 2006; Emery *et al*., 2000). This type of infection does not typically begin to show symptoms until the fruit

start to ripen. Harvested fruit also remain susceptible to brown rot and can be a major source of yield loss if not controlled.

Optimal conditions for fruit rot to occur are between 24°C and 27°C with high humidity (Anderson, 1956). As the fungus begins to develop within the fruit a visible brown lesion forms on the surface of the skin and eventually spreads to the entire fruit. Conidial tufts that look like brownish grey masses begin to develop within the boundaries of the lesion. The fungus continues to develop until the entire fruit is covered by a lesion that is covered by spores. This process can take place in just 5 days in hot, humid, rainy summer conditions. These sporulating areas of infected fruit are easily disseminated by wind, rain and insects over the remainder of a season. Since peach varieties are harvested at different intervals beginning in mid July, each cultivar becomes a source of inoculum for a later cultivar (Hong *et al*., 1997).

A fruit that is fully discolored and wrinkled from infection is referred to as a mummy. In some instances mummies fall to the ground. In other cases, the pathogen mycelia invade the peduncle tissues and prevent the formation of the abscission layer. As a result the fruit will not detach from the tree and remain on the tree throughout the winter. The fungus overwinters as mycelium in both attached and fallen mummies. These mummies are responsible for blossom blight and twig canker development that began the cycle.

1.3 Disease Control

Brown rot is controlled by using efficient orchard sanitation and chemical control. Pruning is a crucial practice because it opens up the canopy and allows for faster drying after a rain. This reduces the humidity that can develop in the microclimate of the

canopy. Weed control can serve as a good sanitation practice because it is likely the fewer weeds in an orchard results in fewer insects. Insect damage can increase crop yield loss by increasing brown rot incidence earlier in a season.

Blossom blight is controlled by early season sprays beginning at bloom. These sprays help prevent twig canker formation as well. Multiple preharvest sprays, which occur from mid July to mid September in New Jersey, are applied to control brown rot throughout the harvest season. Depending on the cultivar, peach fruit will be harvested as early as mid July and as late as mid September. In order to control fruit rot, a number of sprays are needed during this preharvest time period. Benzimidazole fungicides were used to control brown rot up until the 1980's (Zehr *et al*., 1999). Due to resistance developing to the benzimidazoles at this time, demethylation inhibitor or DMI fungicides, such as propiconazole and tebuconazole, have efficiently controlled brown rot for over twenty years (Schnabel *et al*., 2004). Extensive use of DMI fungicides has lead to resistance, recently been reported in the southeastern United States (Schnabel *et al*., 2004).

The most efficient substitutes for DMIs are the quinone outside inhibitors (Q_oI) or strobilurin fungicides. Strobilurins are a relatively new class of fungicides with a mode of action that inhibits mitochondrial respiration. This is achieved by the active ingredient binding at the Q_o site of cytochrome β (Bartlett *et al*., 2002). This specific action inhibits electron transfer, disrupting the cycling of energy within the fungal membrane and blocking the production of ATP. Q_oIs have been shown to control a wide range of diseases on various crops acting as a curative and protective material (Bartlett *et al*., 2002).

There are three main strobilurins registered for use on peach: trifloxystrobin, azoxystrobin and pyraclostrobin. On peach, azoxystrobin and pyraclostrobin are labeled for control of blossom blight and brown rot, where as trifloxystrobin is technically only labeled for control of blossom blight, but can be applied up until one day preharvest and has been recommended for control of brown rot in New Jersey and Ohio (Hamilton *et al*., 2009). There is limited information on the effect of these chemicals on the various monocyclic components of the brown rot infection cycle. Infection, sporulation and dissemination make up the three major components of a pathogen life cycle. Germination, penetration and colonization are part of the infection phase. Sporophore production, spore production and maturation are all part of the sporulation phase. Spore liberation, spore dispersal and spore deposition are part of the dissemination phase.

It is important to know the potential effect of the Q_oI fungicides on each monocyclic component, in order to determine how to best use these fungicides. Protectant activity, resulting from the inhibition of spore germination, has been most commonly studied. However, little information is known about what effects the strobilurins have on colonization. Since the life cycle of brown rot is polycyclic and many spores are produced over one season, it is also important to determine the effect of these fungicides on the sporulation phase.

In our studies, these three strobilurins were examined at different rates and application timings to determine there effectiveness at inhibiting colonization and sporulation of twig cankers and fruit rot. As the threat of DMI resistance looms in the Southeastern part of the United States, it is important to begin anticipating and changing

current control methods. The first step in this process is to understand the properties of the strobilurins and when is the best time to use them.

2. EFFECT OF QOI FUNGICIDES ON COLONIZATION AND SPORULATION OF *MONILINIA FRUCTICOLA* ON PEACH FRUIT AND BLOSSOM BLIGHT CANKERS

2.1 Introduction

Brown rot is a major disease on peach (*Prunus persica* (L.) Batsch.) and other stone fruits in North America and throughout the world. Conidial inoculum, principally from overwintering mummified fruit, infects flowers in early spring, resulting in blossom blight and the formation of twig cankers. Spores produced on these blighted flowers and cankers infect fruit during the pre-harvest ripening period (Watson *et al*., 2002). Diseased fruit produce secondary inoculum for further fruit infection within the same cultivar and for subsequent infections on later cultivars. Thus, fruit rot can be the cause for major crop loss if proper control is not provided during bloom and the entire ripening period from the first to the last cultivar harvested.

In the Eastern United States and other wet climates, the most effective method for brown rot control is application of fungicides (Agrios, 1997; Zehr, 1982). In the past, the demethylation inhibitor (DMI) fungicides were the most commonly applied protectant fungicides. One or more sprays were applied during bloom to prevent blossom blight and twig canker formation, and two to three applications were typically applied during the pre-harvest period to control fruit rot (Agrios, 1997; Holb and Schnabel, 2006; Luo and Schnabel, 2008a). However, since fruit on the various cultivars ripen over approximately a two-month period, many consecutive applications of DMI fungicides were often applied. For example, if harvests were obtained from cultivars in eight maturity groups, then as many as 16 to 24 fungicide applications were utilized per season

for preharvest sprays alone. Such extensive use of DMI fungicides has resulted in the development of resistant strains of *M. fructicola* (Luo and Schnabel, 2008b; Cox *et al*., 2007).

Strobilurin fungicides, also known as quinone outside inhibitors or Q_oI's, became available for use on a wide variety of crops in 1996. Since then they have been incorporated into spray programs for many crops (Bartlett *et al*., 2002). Q_oI activity is directed at inhibiting mitochondrial respiration, more specifically binding at the Q_o site of the cytochrome bc_1 complex (Bartlett *et al*., 2002). Since this alternative mode of action is distinctly different from the DMI fungicides, cross resistance is unlikely between the two fungicide classes. Thus, incorporation of Q_oI's into the brown rot management program can be an important resistance management strategy. Indeed, in a previous study it has been suggested that Q_oI fungicides would make a viable alterative or rotation partner for control of brown rot in peach orchards (Schnabel *et al*., 2004).

The Q_oI fungicides have been shown to have protective and curative properties (Bartlett *et al*., 2002). On *Beta vulgaris* L. cv Rizor , the strobilurin fungicides trifloxystrobin and pyraclostrobin have been shown to completely inhibit spore germination of *Cercospora beticola*, preventing Cercospora leaf spot (Karadimos *et al*., 2005). Azoxystrobin has been shown to have curative effects against grapevine downy mildew and other pathosystems by causing mycellial collapse (Bartlett *et al*., 2002; Wong and Wilcox, 2001). Azoxystrobin exhibited better protective and curative activities than trifloxystrobin against downy mildew of pearl millet by providing better anti-sporulant activity and showing evidence of root uptake and translaminar movement (Sudisha *et al*., 2005). Azoxystrobin exhibited curative activity against Septoria blotch on wheat by

reducing the rate of growth of intercellular hyphae of *Mycosphaerella graminicola* (Rohel *et al*., 2001). Trifloxystrobin provided good anti-sporulant activity and restricted development of *Cladosporium fulvum,* causal agent of tomato leaf mold (Veloukas *et al*., 2007). Similarly, trifloxystrobin significantly reduced sporulation of a related pathogen, *Fusicladosporium carpophilum*, on peach twig lesions (Lalancette *et a*l., 2008). Finally, pyraclostrobin, when mixed with epoxiconazole, prevented sporulation of *Quambalaria eucalypti*, which causes leaf spot and shoot curl on eucalyptus (Ferreira *et al*., 2008). These examples demonstrate that Q_oIs can provide significant curative as well as protectant activity in a number of different pathosystems. However, little or no information is available on the curative activity of Q_oIs against *M. fructicola* on stone fruit.

The overall objective of our study was to quantify the inhibitory effects of Q_oI fungicides against *M. fructicola* colonization and sporulation on peach fruit. Specifically, our goals were to determine if fungicide application timing (relative to time of infection) and fungicide rate had an impact on these two monocyclic components of the infection cycle. Furthermore, because of their importance as an early-to-mid season inoculum source, the anti-sporulant activity of the Q_oIs against blossom blight cankers was also examined. Finally, in each of these studies, comparisons were made across those Q_oI fungicides currently registered for peach. Preliminary findings on various aspects of this research have been previously reported (Burnett *et al*., 2006c; Burnett *et al*., 2007b; Burnett *et al*., 2007a; Lalancette *et al.,in press*).

2.2 Materials and Methods

2.2.1 Orchard site. Two experiments were conducted in a mixed cultivar orchard located at the Rutgers Agricultural Research and Extension Center, Bridgeton. The orchard was planted in 1996 with a tree x row spacing of 6.1 m x 7.6 m. The cultivars were 'Autumnglo' peach, 'Suncrest' peach, and 'Redgold' nectarine, each grafted on 'Lovell' rootstock. Standard commercial practices for tree management, insect control, and weed control were followed throughout the experiments (Hamilton *et al*., 2008).

2.2.2 Experimental design. Each experiment was arranged in a randomized complete block design with four single tree replicates of either 'Autumnglo' or 'Suncrest' peach trees. Fungicide treatments were applied by a Rears Pak-Blast-Plot airblast sprayer (Rears manufacturing, Eugene, OR) at 935L/ha and 689.5 kPa during the fruit ripening period approximately 21 to 14 days preharvest. The fungicides examined in the experiments were azoxystrobin at 280.2 g/ha (Abound 2.08F; Syngenta Crop Protection, Greensboro, NC); trifloxystrobin at 140.1 g/ha (Flint 50WG; Bayer CropScience, Research Triangle Park, NC); and pyraclostrobin + boscalid at 130.0 + 256.8 g/ha, respectively (Pristine 38WG; BASF Corp., Research Triangle Park, NC). These rates were the maximum commercial rates listed for peach on the current product labels. In both studies, treatment trees were surrounded on all sides by non-sprayed buffer trees to reduce inter-plot interference from spray drift.

2.2.3 Fruit inoculation. Isolates of *M. fructicola* were collected from naturally infected peaches found in commercial orchards located in southern New Jersey. To produce inoculum, these isolates were grown on canned peach halves for seven days in

an incubator set at 25°C. Conidia were harvested by washing off the colonies with deionized water using a DeVilbiss atomizer set at 34.5 kPa (DeVilbiss Health Care, Somerset, PA). Collected spores were counted using a hemacytometer and diluted to a concentration of 10,000 conidial/ml. Twenty attached fruit per tree were injected with 0.5 ml conidial suspension to an approximate depth of 4 mm below the epidermis using a 10 ml syringe needle. Fruit were removed from the tree one to two days post inoculation and were placed in trays in a greenhouse, held between 25-30°C, for the remainder of the incubation period.

2.2.4 Autumnglo study. Treatments in the Autumnglo study, conducted during 2005, 2006 and 2007 preharvest periods, consisted of a 3 x 3 factorial. The first factor was fungicide type which consisted of azoxystrobin, trifloxystrobin, or pyraclostrobin+boscalid. The second factor, inoculation timing, was comprised of the following levels: (i) spray then inoculate 24 h later (SI); (ii) inoculate then spray 24 h later (IS); and (iii) spray twice, at a seven day interval, then inoculate 24 h after the second spray (SSI). Since the SSI fruit were inoculated 7 days after the SI and IS fruit, separate control treatments were used for the two different inoculation timings. Control fruit were inoculated but not treated with fungicide. The SSI factor level was not examined in 2006.

2.2.5 Suncrest study. Treatments in the Suncrest study, conducted during the 2006 and 2007 preharvest periods, consisted of a 2 x 2 x 2 factorial. The first factor was fungicide type (Fung), which consisted of either azoxystrobin or trifloxystrobin. The second factor was a combination of volume and rate (VolRate). The 1X treatment level had the maximum labeled rate of fungicide applied at 935L/ha. The 2X treatment level

had double the maximum labeled rate applied at double the spray volume or 1,871L/ha. The third factor was timing of inoculation relative to fungicide application (Timing). Treatment levels consisted of either immediate inoculation (II) or delayed inoculation (DI) performed at 24 h or 7 days after fungicide application, respectively. Separate controls were used for the two different inoculation timings. Control fruit were inoculated but not treated with fungicide.

2.2.6 Disease assessment. During each assessment, the short and long diameters of the lesion and sporulating areas on each inoculated fruit were measured using a flexible rule. Areas for these regions were calculated using the formula for an ellipse where the short and long diameters represented the minor and major axes of the ellipse, respectively. Disease assessments in the 'Autumnglo' study in 2005 were conducted at 3, 4, and 6 days post inoculation; in 2006 and 2007, assessments were performed at 5 and 7 days post inoculation. Disease assessments in the 'Suncrest' study were taken at 5 and 7 days post inoculation in 2006 and 2007.

2.2.7 Statistical analysis. Two dependent variables, area under the colonization curve (AUCC) and area under the sporulation (AUSC) curve, were calculated in both studies for the period between the final two assessments. An equivalent two-day period elapsed between these assessments, thereby allowing treatment comparisons across all years. To determine treatment efficacy, AUCC and AUSC values were compared to their respective controls using Dunnett's one-tailed t-test. Since fruit susceptibility increases with maturation and ripening, SSI and DI factor levels in the Autumnglo and Suncrest studies could not be directly compared with the IS/SI and II levels, respectively. Consequently, to adjust for changing fruit susceptibility, relative AUCC and AUSC

values were calculated as $AUCC_r = AUCC_{trt}/AUCC_{ctl}$ and $AUSC_r = AUSC_{trt}/AUSC_{ctl}$, where the "r" subscript denotes relative area values, the "trt" subscript denotes individual treatment values, and the "ctrl" subscript represents the respective nontreated control value. Analyses of variance were then performed on these relative areas and main effects means were compared using Tukey's HSD test. Finally, specific treatment means for $AUCC_r$ and $AUSC_r$ were compared using the Waller-Duncan *k*-ratio *t*-test. All analyses were conducted using the Statistical Analysis System version 9.1 (SAS Institute, Inc., Cary, NC).

2.2.8 Canker study. Insufficient numbers of blossom blight cankers were formed during the 2005-2007 seasons to allow adequate replication for an experiment. However, in 2008 high levels of cankers were observed in an 'Autumnglo' peach block. During the summer, a total of 16 trees harboring large numbers of cankers were selected in the block. Four trees were randomly assigned to each of three fungicide treatments plus a control in a completely randomized design (n = 4).

On each treatment tree, fifteen shoots having one or more cankers were tagged. Cankers were then washed with water using pressurized hand sprayers to remove any conidia already formed. Immediately after drying, azoxystrobin at 0.150 g/L, trifloxystrobin at 0.074 g/L, and pyraclostrobin + boscalid at 0.069 g/L + 0.136 g/L were applied until run-off to the shoots using hand atomizers; control shoots received no treatment. After 7 days of field exposure, the tagged shoots were cut from the trees, placed in plastic bags, and stored overnight in a refrigerator at 4.5°C.

Twigs from each treatment tree were placed in separate covered trays containing moist paper towels to provide RH >95%. Trays were then put in an incubator set at a

constant 22°C; after 24 h, the covers were removed and the trays allowed to incubate for another 24 h. Following incubation, twig cankers were first assessed for presence of sporulation using a stereoscopic microscope at 50X. Twig canker length was then measured using a rule. Finally, conidia were harvested with water using a DeVilbiss atomizer at 34.5 kPa (DeVilbiss Health Care, Inc., Somerset, PA); conidial numbers were estimated using a hemacytometer. From these data, dependent variables were percentage of sporulating cankers, #conidia/canker, and #conidia/mm canker length. The entire experiment was repeated one time.

2.3 Results

2.3.1 Autumnglo study. Based on visual examination of lesion growth curves, both fungicide type and inoculation timing factors appeared to influence expansion of the sporulating area (Fig 2.1) In general, the greatest inhibition of sporulating area was observed with the SSI and trifloxystrobin treatments. This inhibition was evident in 2005 and 2006, when most sporulating areas were below 30 cm^2, as well under more favorable colonization conditions in 2007, when some sporulating areas exceeded 100 cm^2.

Separate analyses of variance performed on the IS/SI and SSI data sets showed that the year x fungicide interaction was not significant for AUCC ($P \geq 0.55$) but was significant for AUSC ($P \geq 0.005$). These results indicated that the yearly data sets could be pooled prior to analysis for the AUCC variable. However, since such data pooling was not possible for AUSC, data for both variables were analyzed separately by year.

The Q_oI treatments had a small, but consistent effect on *M. fructicola* colonization of fruit. Over the three-year period, 22 of the 24 treatments examined (92%) had numerically lower AUCC levels than their respective controls (Table 2.1). Of these 22

treatments, 6 or 27% had significantly lower AUCC means than the controls. Azoxystrobin provided the most consistent reduction in lesion development, significantly reducing the AUCC in 3 of the 8 treatments or 38% of the time. However, the magnitudes of these reductions by azoxystrobin were not large; AUCC's were decreased by only 10.8 to 12.1%. In contrast to AUCC, only 13 of the 24 treatments (54%) had numerically lower AUSC levels than their respective nontreated controls (Table 2.1). Nevertheless, all eight of the trifloxystrobin treatments had numerically lower AUSC's, of which three were significantly less than the control. Furthermore, these treatments reduced sporulating area to a much greater extent than observed with colony area. The trifloxystrobin IS and SI treatments reduced AUSC's by 39.6 to 45.2%, while the SSI treatment provided a 54.8 to 63.1% reduction.

Analysis of variance of relative growth data showed that fungicide type had no effect on $AUCC_r$ ($P \geq 0.34$) but a highly significant effect on $AUSC_r$ ($P \leq 0.0029$) (Table 2.2). Hence, the fungicide main effects means were not significantly different for $AUCC_r$ (Fig. 2.2A), while trifloxystrobin exhibited significantly lower $AUSC_r$'s than pyrac+bosc in all three years and azoxystrobin in two of three years (Fig. 2.2C). In contrast, results of analysis of variance for inoculation timing were somewhat more variable (Table 2.2). This main effect was significant twice for $AUCC_r$, in 2005 and 2007, and only once for $AUSC_r$, in 2007. No consistent separations in main effects means were observed across years for $AUCC_r$ (Fig. 2.2B). However, the SSI timing exhibiting significantly lower $AUSC_r$'s in both years that it was examined (Fig. 2.2D).

The most effective treatment combinations for reducing colony area in 2005, 2006, and 2007 were pyrac+bosc/SSI, pyrac+bosc/IS, and azoxystrobin/SI, respectively

(Table 2.3). In general, $AUCC_r$ treatment means were somewhat lower for either pyrac+bosc or azoxystrobin, while trifloxystrobin reductions tended to be less. However, since overall reductions by treatments were not large, few significant differences were observed. In contrast, $AUSC_r$ treatment means for trifloxystrobin SSI and SI were significantly lower than corresponding pyrac+bosc means in all three years, indicating that trifloxystrobin provided better antisporulant activity. Although $AUSC_r$'s for all trifloxystrobin treatment combinations were numerically lower than those for azoxystrobin, significant differences between these two fungicides were only observed for the SI and SSI treatments in 2007.

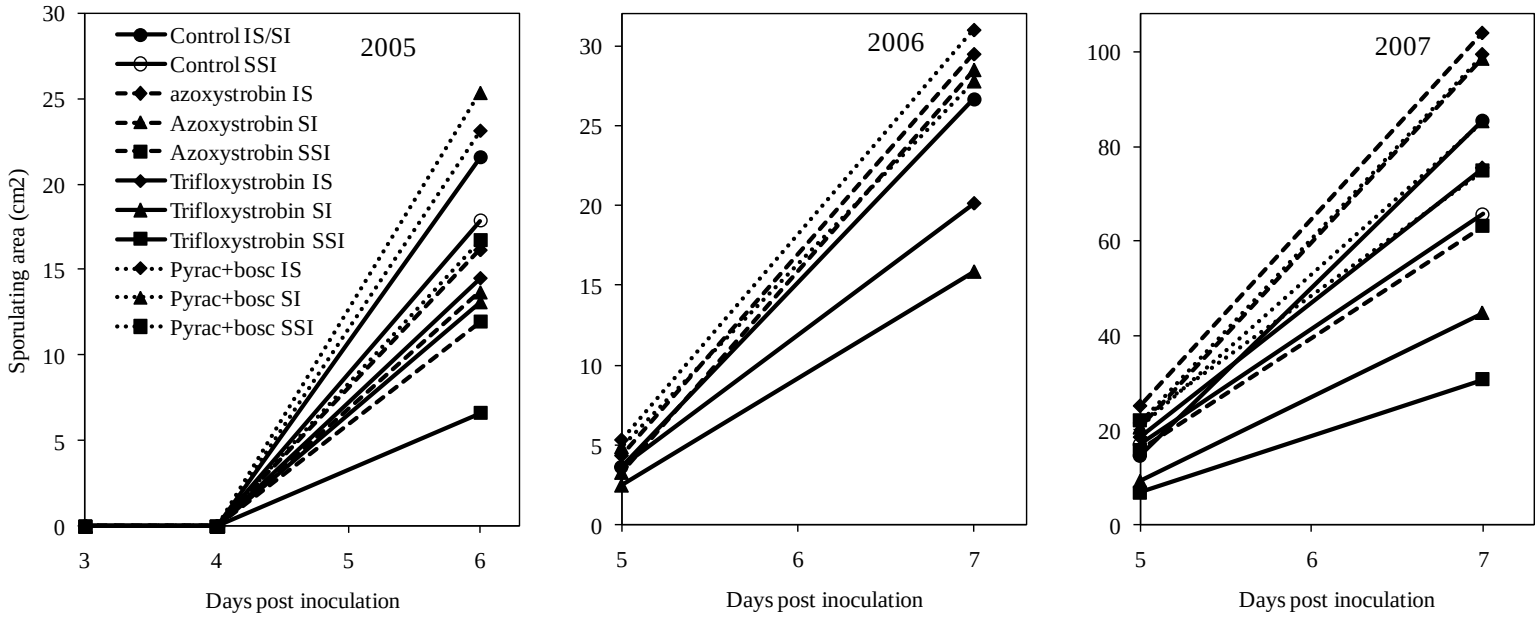

Figure 2.1. Influence of strobilurin treatments on growth of sporulating area of *M. fructicola* on inoculated 'Autumnglo' fruit during the pre-harvest fruit ripening period in August 2005, 2006, and 2007. Inoculation timings were: SI = spray then inoculate 24 h later; IS = inoculate then spray 24 h later; and SSI = spray twice, at a seven day interval and then inoculate 24 h after the second spray. Control fruit were inoculated but not sprayed with fungicide.

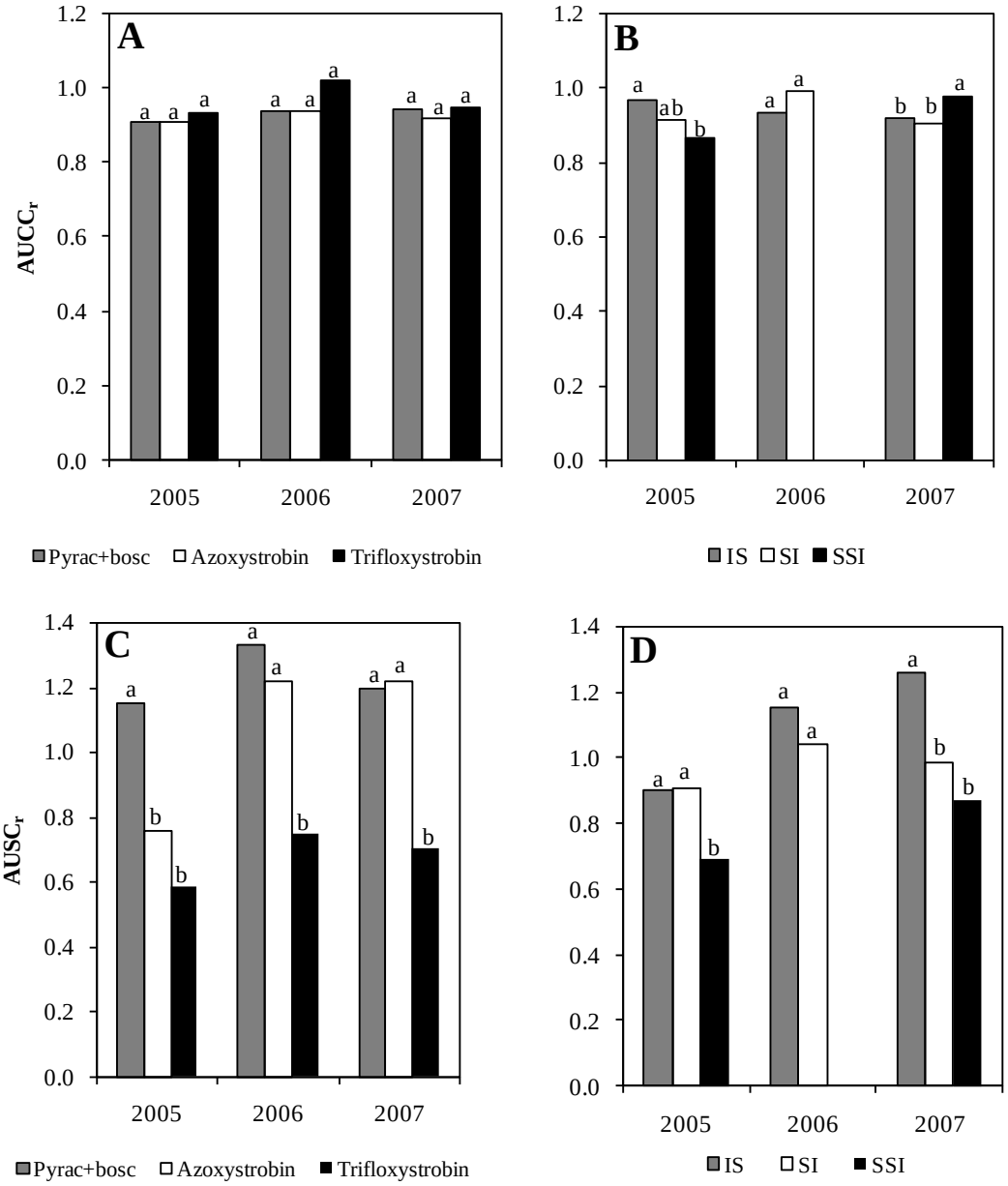

Figure 2.2. Comparison of the fungicide and inoculation timing main effect means for relative area under the colonization (A,B) and sporulation (C,D) curves for 'Autumnglo' fruit inoculated with *M. fructicola*. Inoculation timings were: SI = spray then inoculate 24 h later; IS = inoculate then spray 24 h later; and SSI = spray twice, at a seven day interval, then inoculate 24 h after the second spray. Means within each year having the same letter are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

Table 2.1. Comparison of strobilurin treatments to non-treated control for area under the colonization (AUCC) and sporulation (AUSC) curves on 'Autumnglo' peach fruit inoculated with *M. fructicola.*

		AUCC[a]			**AUSC[a]**		
Fungicide	Inoc. timing[b]	2005	2006	2007	2005	2006	2007
Pristine	IS	71.8	122.8	265.5	23.2	36.6	121.6
	SI	70.7	134.1	254.2*	25.4	32.9	114.7
	SSI	82.6*	…	255.7	16.7	…	97.0
Abound	IS	71.8*	123.1	253.4*	16.2	34.3	129.4
	SI	66.9	135.4	252.1*	13.7	31.6	119.7
	SSI	86.9*	…	250.6	12.0	…	78.4
Flint	IS	76.3	141.1	262.7	14.4	23.7	94.0
	SI	70.2	143.7	262.2	13.2	18.3	54.5*
	SSI	84.2*	…	254.2	6.63*	…	37.6*
Control	IS/SI	76.1	142.9	284.1	21.9	30.3	99.4
Control	SSI	97.8	…	259.0	17.9	…	3.2

[a] Areas calculated over a two-day period between 4- and 6-days post inoculation (2005) or 5- and 7-days post inoculation (2006-2007). Treatment means significantly different from their respective controls are indicated by an asterisk according to Dunnetts t-test (α=0.05).

[b] SI = spray then inoculate 24 h later; IS = inoculate then spray 24 h later; and SSI = spray twice, at a seven day interval, then inoculate 24 h after the second spray. Control fruit were inoculated but not sprayed with fungicide.

Table 2.2. Analysis of variance of relative area under the colonization ($AUCC_r$) and sporulation ($AUSC_r$) curves for treatments applied to 'Autumnglo' peach fruit inoculated with *M. fructicola*.

			$AUCC_r$[a]			$AUSC_r$[a]		
Year[b]	Source	df	MS	*F* Value	*P* > *F*	MS	*F* Value	*P* > *F*
2005								
	Model	11	0.2944	2.78	0.0174	7.0710	4.53	0.0010
	Rep	3	0.5413	5.12	0.0070	9.5573	6.12	0.0031
	Fungicide	2	0.0399	0.38	0.6900	20.1202	12.88	0.0002
	Timing	2	0.6333	5.99	0.0078	3.6690	2.35	0.1171
	Error	24	0.1058	…	…	1.5624	…	…
2006								
	Model	8	1.7993	4.39	0.0066	14.0229	8.54	0.0002
	Rep	3	4.3287	10.57	0.0005	27.2697	16.61	<.0001
	Fungicide	2	0.3733	0.91	0.4231	14.4680	8.81	0.0029
	Timing	1	0.3907	0.95	0.3442	1.3903	0.85	0.3720
	Error	15	0.4096	…	…	1.6414	…	…
2007								
	Model	11	0.2134	4.96	0.0005	7.5883	6.1	0.0001
	Rep	3	0.5013	11.65	<.0001	6.3747	5.12	0.0070
	Fungicide	2	0.0485	1.13	0.3402	20.0114	16.07	<.0001
	Timing	2	0.3529	8.20	0.0019	8.7006	6.99	0.0041
	Error	24	0.0430	…	…	1.2449	…	…

[a]Relative areas calculated over a two-day period between 4- and 6-days post inoculation (2005) or 5- and 7-days post inoculation (2006-2007).

[b]Analyses in 2005 and 2006 performed on all inoculation timings (IS, SI and SSI); analysis in 2006 performed only on IS and SI timings.

Table 2.3. Comparison strobilurin treatments for relative areas under the colonization ($AUCC_r$) and sporulation ($AUSC_r$) curves on 'Autumnglo' peach fruit inoculated with *M. fructicola*.

Treatment		$AUCC_r$[a]			$AUSC_r$[a]		
Fungicide	timing[b]	2005	2006	2007	2005	2006	2007
Pyrac+bosc	IS	0.95 ab	0.88 a	0.93 abc	1.20 ab	1.39 a	1.29 ab
	SI	0.93 ab	0.98 a	0.90 bc	1.29 a	1.28 ab	1.10 ab
	SSI	0.85 b	…	0.99 a	0.97 abc	…	1.20 ab
Azoxystrobin	IS	0.95 a	0.90 a	0.90 c	0.81 bcd	1.27 ab	1.42 a
	SI	0.88 ab	0.97 a	0.89 c	0.77 bcd	1.16 abc	1.28 ab
	SSI	0.89 ab	…	0.97 abc	0.70 cd	…	0.94 bc
Trifloxystrobin	IS	1.00 a	1.02 a	0.93 abc	0.69 cd	0.81 bc	1.06 ab
	SI	0.93 ab	1.02 a	0.93 abc	0.67 cd	0.68 c	0.59 cd
	SSI	0.86 b	…	0.98 ab	0.40 d	…	0.47 d

[y] Relative areas calculated over a two-day period between 4- and 6-days post inoculation (2005) or 5- and 7-days post inoculation (2006-2007). Means in the same column followed by the same letter are not significantly different according to Waller-Duncan *k*-ratio *t*-test ($\alpha = 0.05$; $k = 100$)

[z] SI = spray then inoculate 24 h later; IS = inoculate then spray 24 h later; and SSI = spray twice, at a seven day interval, then inoculate 24 h after the second spray.

2.3.2 Suncrest study. Sporulating areas on untreated control fruit in 2006 increased at a rate of 3.0-3.6 cm^2/day, reaching 21.2-25.4 cm^2 by 7-days post inoculation (Fig. 2.3). In 2007, untreated sporulating areas increased at 10.3-10.8 cm^2/day, attaining 71.9-75.9 cm^2 by day seven. The more rapid lesion development observed in 2007 was most likely due to a higher degree of fruit maturity, and therefore susceptibility, at time of inoculation (Hall, 1972). Regardless of this varying susceptibility across years, all trifloxystrobin treatments consistently reduced sporulating area to a greater extent than their corresponding azoxystrobin treatments. Furthermore, in both years the 2X trifloxystrobin treatment growth curves were consistently lower than the 1X trifloxystrobin treatments. However, this pattern was not evident for the azoxystrobin treatments.

In 2006, all treatments significantly lowered the AUCC, and all but azoxystrobin 1X/II also significantly lowered the AUSC (Table 2.4). These results indicated that under conditions less favorable to lesion development, pathogen growth and sporulation were inhibited regardless of fungicide type, rate of application, or inoculation timing. In comparison, the more pathogen favorable conditions in 2007 resulted in only one trifloxystrobin and two azoxystrobin treatments having lower AUCC's than the untreated controls (Table 2.4). Nevertheless, even under these favorable conditions, all four trifloxystrobin treatments significantly reduced growth of the sporulating areas based on the AUSC values. The greatest inhibition was observed for the 2X/II treatment, which provided a 67.4% reduction in sporulating area at 7-days post inoculation.

Results of the analysis of variance indicated that the fungicide main effect was significant for both $AUCC_r$ and $AUSC_r$ in both years of the study (Table 2.5). Subsequent

comparison of the main effects means for $AUCC_r$ and $AUSC_r$ showed that although azoxystrobin provided significantly better control of colonization, trifloxystrobin had a significantly greater ability to reduce growth of the sporulating area (Fig. 2.4A,D). These effects were consistent across the less disease-favorable conditions encountered in 2006 and the more disease-favorable conditions in 2007. Interestingly, doubling the spray volume and rate only significantly lowered the $AUCC_r$ in 2006, although this factor was nearly significant ($P = 0.06$) for the $AUSC_r$ dependent variable in both years (Table 2.5, Fig. 2.4B,E). Although delayed inoculation tended to lower the $AUSC_r$ (Fig 2.4F), the timing factor was significant in only one of the two years (Table 2.5). None of the two-way interactions between the main effects were significant in either year.

Examination of the specific treatment means showed that the azoxystrobin 2X treatments were the most efficient at controlling lesion development (Table 2.6). In 2006, the azoxystrobin 2X/II treatment provided the greatest effect, reducing the $AUCC_r$ by 46%, while the best trifloxystrobin treatment, 2X/II, only reduced the $AUCC_r$ by 24%. In 2007, a similar trend occurred, although most treatments were not significantly different from each other; maximum $AUCC_r$ reductions were 18% for azoxystrobin 2X/DI and 7% for trifloxystrobin 2X/II. Examination of specific treatment means for $AUSC_r$ showed that both 1X and 2X trifloxystrobin treatments were equally effective at reducing the sporulating area, although all 2X treatments were numerically lower than their corresponding 1X treatments (Table 2.6). Maximum control of sporulating area occurred in less disease favorable 2006, when trifloxystrobin 2X/DI and "label-rate" 1X/DI treatments reduced $AUSC_r$ by 98% and 89%, respectively. Reductions in $AUSC_r$

by azoxystrobin were relatively lower than those observed for trifloxystrobin, although some treatment overlap occurred.

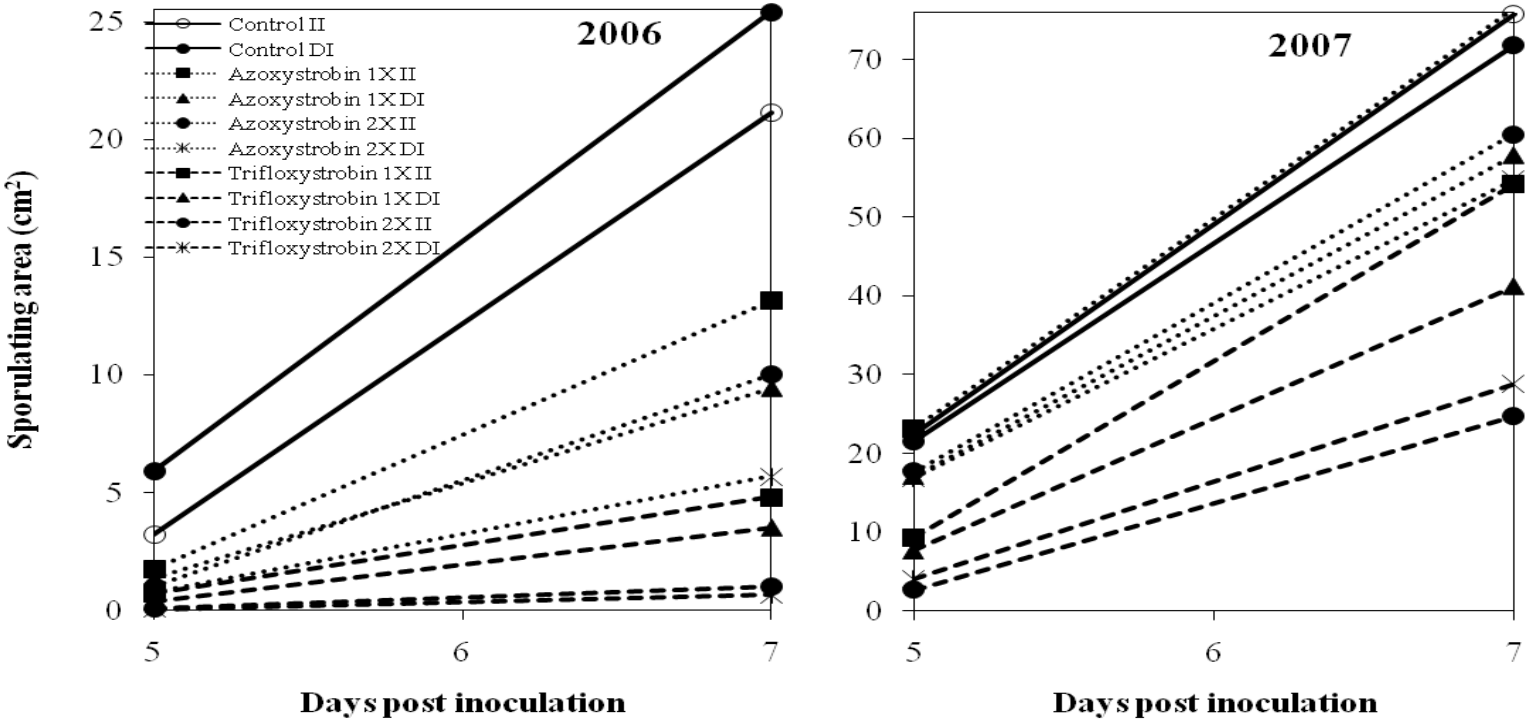

Figure 2.3. Influence of strobilurin treatments on growth of sporulating area of *M. fructicola* on inoculated 'Suncrest' fruit during the pre-harvest fruit ripening period in August 2006 and 2007. 1X = maximum labeled rate at 935L/ha volume; 2X = double the maximum rate at double the volume of 1,871L/ha; II = immediate inoculation after spray; and DI = delayed inoculation, at 7-days post spray. Control fruit were inoculated but not sprayed with fungicide.

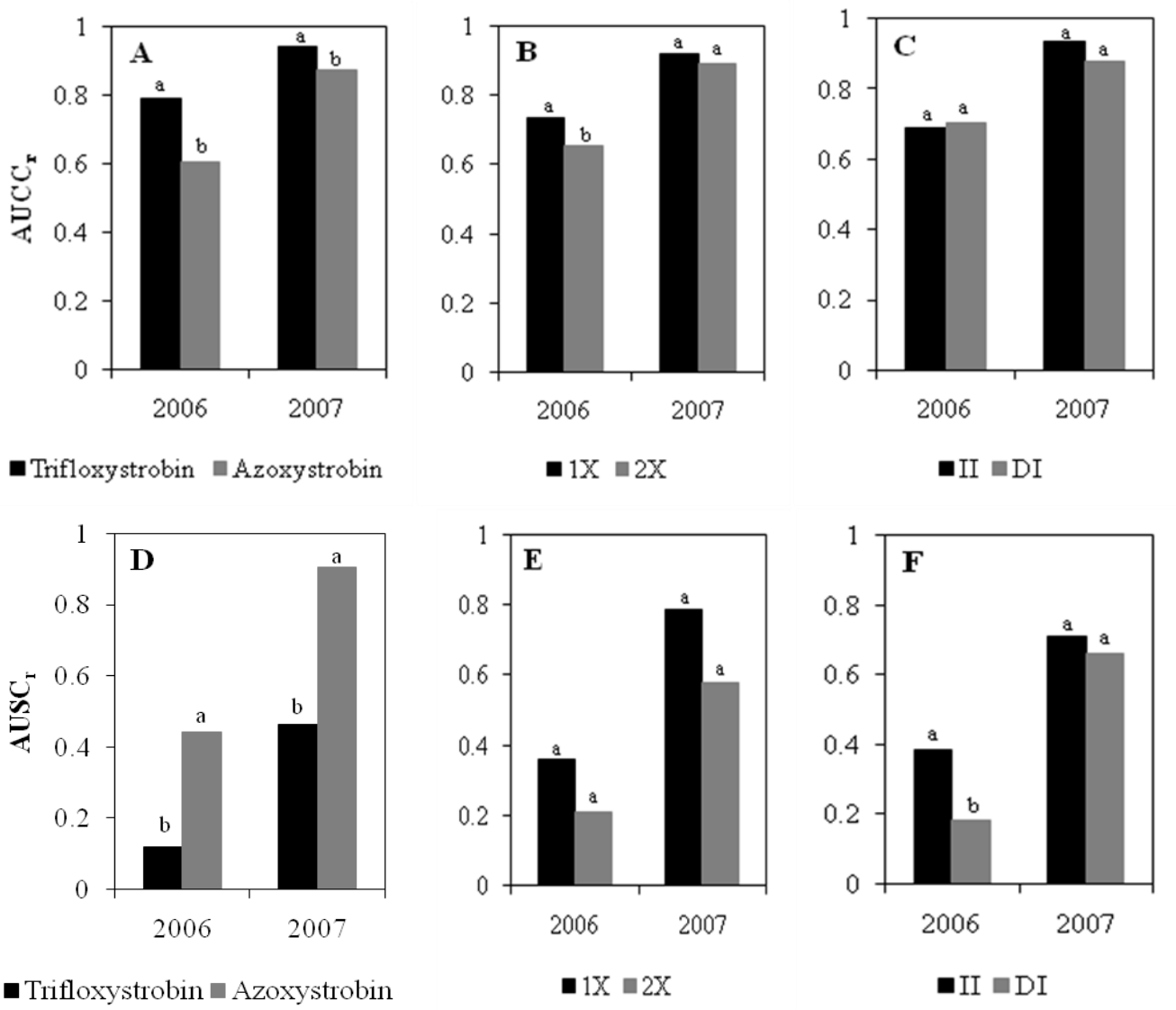
A
B
C
D
E
F
AUCC$_r$
AUSC$_r$
1
0.8
0.6
0.4
0.2
0
2006
2007
a
b
Trifloxystrobin
Azoxystrobin
1X
2X
II
DI

Figure 2.4. Comparison of fungicide, application rate/volume, and inoculation timing main effect means for relative area under the colonization (A-C) and sporulation (D-F) curves for 'Suncrest' fruit inoculated with *M. fructicola*. Rate/volume levels: 1X = maximum labeled rate at 935L/ha volume; and 2X = double the maximum rate at double the volume of 1,871L/ha. Inoculation timing levels: II = immediate inoculation after spray; and DI = delayed inoculation, at 7-days post spray. Means within each year having the same letter are not significantly different according to Tukey's HSD test ($\alpha = 0.05$).

Table 2.4. Comparison of strobilurin treatments to nontreated controls for area under the colonization (AUCC) and sporulation (AUSC) curves on 'Suncrest' peach fruit inoculated with *M. fructicola*.

		Inoc.	AUCC[a]		AUSC[a]	
Fungicide	Rate[c]	Timing[b]	2006	2007	2006	2007
Azoxystrobin	1X	II	63.5*	251.2	15.3	100.2
		DI	67.2*	214.5	10.8*	75.3
	2X	II	49.0*	227.9*	10.9*	80.6
		DI	56.4*	196.6*	6.5*	71.9
Trifloxystrobin	1X	II	72.7*	265.2	5.4*	63.4*
		DI	84.0*	211.0*	3.6*	49.9*
	2X	II	70.8*	265.4	1.0*	27.5*
		DI	81.1*	222.4	0.7*	32.7*
Control	…	II	93.2	271.0	24.2	98.8
Control	…	DI	101.9	240.0	31.4	92.0

[a] Areas calculated over a two-day period between 5- and 7-days post inoculation. Treatment means significantly different from their respective controls are indicated by an asterisk (*) according to Dunnetts t-test ($\alpha = 0.05$).

[b] II = immediate inoculation after spray; DI = delayed inoculation, at 7-days post spray. Control fruit were inoculated but not sprayed with fungicide.

[c] 1X = maximum labeled rate at 935L/ha volume; 2X = double the maximum rate at double the volume of 1,871L/ha.

Table 2.5. Analysis of variance of relative areas under the colonization ($AUCC_r$) and sporulation ($AUSC_r$) curves for treatments applied to 'Suncrest' peach fruit inoculated with *M. fructicola*.

			$AUCC_r$[a]			$AUSC_r$[a]		
Year	Source	df	MS	*F* value	*P* > *F*	MS	*F* value	*P* > *F*
2006	Model	9	0.9044	6.25	0.0002	3.3604	4.15	0.0031
	Block	3	0.6352	4.39	0.0145	1.4812	1.83	0.1711
	Fungicide	1	5.0012	34.58	<.0001	14.1704	17.51	0.0004
	VolRate	1	0.8876	6.14	0.0214	3.1765	3.93	0.0602
	Timing	1	0.0270	0.19	0.6697	5.8340	7.21	0.0135
	Fung x VolRate	1	0.3210	2.22	0.1505	0.0331	0.04	0.8416
	Fung x Timing	1	0.0447	0.31	0.5838	1.4048	1.74	0.2012
	VolRate x Timing	1	0.0112	0.08	0.7833	0.2649	0.33	0.5730
	Error	22	0.1446			0.8091		
2007	Model	9	0.2647	1.99	0.0911	4.7910	3.16	0.0134
	Block	3	0.1693	1.27	0.3089	1.4329	0.94	0.4365
	Fungicide	1	0.7110	5.34	0.0306	29.1993	19.23	0.0002
	VolRate	1	0.1261	0.95	0.3411	5.9835	3.94	0.0597
	Timing	1	0.4124	3.10	0.0924	0.5036	0.33	0.5705
	Fung x VolRate	1	0.4391	3.30	0.0831	0.9244	0.61	0.4435
	Fung x Timing	1	0.0991	0.74	0.3977	0.6006	0.4	0.5358
	VolRate x Timing	1	0.0322	0.24	0.6277	1.2975	0.85	0.3653
	Error	22	0.1332			1.5183		

[a]Area under colonization and sporulation curves calculated over a 2-day period between 5- and 7-days post inoculation.

Table 2.6. Comparison of strobilurin treatments for the relative area under colonization ($AUCC_r$) and sporulation ($AUSC_r$) curves on 'Suncrest' peach fruit inoculated with *M. fructicola*.

		Inoc.	$AUCC_r$[x]				$AUSC_r$[x]			
Fungicide	Rate[y]	Timing[z]	2006		2007		2006		2007	
Azoxystrobin	1X	II	0.68	bc	0.93	ab	0.67	a	1.08	a
		DI	0.66	bcd	0.90	ab	0.34	abc	0.86	ab
	2X	II	0.54	d	0.84	ab	0.51	ab	0.86	ab
		DI	0.55	cd	0.82	b	0.23	bc	0.83	abc
Trifloxystrobin	1X	II	0.79	ab	0.98	ab	0.29	bc	0.64	abcd
		DI	0.82	a	0.88	ab	0.11	c	0.56	bcd
	2X	II	0.76	ab	0.98	a	0.05	c	0.27	d
		DI	0.79	ab	0.93	ab	0.02	c	0.37	cd

[x] Areas calculated over a two-day period between 5- and 7-days post inoculation. Means in the same column followed by the same letter are not significantly different according to the Waller-Duncan *k*-ratio *t*-test ($\alpha = 0.05$; $k = 100$).

[y] 1X = maximum labeled rate at 935L/ha volume; 2X = double the maximum rate at double the volume of 1,871L/ha.

[z] II = immediate inoculation after spray; DI = delayed inoculation, at 7-days post spray.

2.3.3 Canker study. The fungicide treatment main effects for all three dependent variables were significant in the analyses of variance (Table 2.7). Conidia production and adjusted conidia production fungicide effects were highly significant ($P < 0.007$), while the treatment effect for sporulation incidence was barely significant ($P = 0.04$). Since all treatment x replication interactions were not significant, fungicide treatment differences did not vary across both experimental replications. Thus, these analyses of variance were performed on the pooled data.

The number of cankers with visible sporulation was significantly reduced by pyraclostrobin + boscalid and trifloxystrobin, but not by azoxystrobin (Table 2.8). The level of reduction in incidence of sporulating cankers ranged from 24% for pyraclostrobin + boscalid to 31% for trifloxystrobin. Although sporulation on most cankers was not completely inhibited, actual conidia production was considerably and significantly reduced by all three fungicides (Table 2.8). The number of conidia per canker was reduced by 54.7%, 60.6%, and 73.3% for pyraclostrobin + boscalid, azoxystrobin, and trifloxystrobin, respectively; after correction for canker size, reductions were 53.1%, 56.1%, and 71.4%, respectively.

Table 2.7. Analysis of variance of sporulation incidence and spore production of *M. fruticola* on 'Autumnglo' peach twig cankers treated with Q_oI fungicides.

		Sporulation Incidence			Conidia Production			Adjusted Conidia Production		
Source [a]	df	MS	*F* value	*P* > *F*	MS	*F* value	*P* > *F*	MS	*F* value	*P* > *F*
Model	7	855.3	6.45	0.0002	9.58E+08	4.61	0.0022	3384043	5.88	0.0005
ExpRep	1	4228.8	31.89	<0.0001	1.93E+09	9.38	0.0054	7182254	12.47	0.0017
Treatment	3	421.6	3.18	0.0422	1.17E+09	5.19	0.0066	3790205	6.58	0.0021
Trt x ExpRep	3	164.5	1.24	0.3169	5.03E+08	2.45	0.0885	1711809	2.97	0.0518
Error	24	132.6			2.16E+08			575877		

[a] Results based on a completely randomized design with four replicate trees per fungicide treatment and two experimental replications (ExpRep). Fifteen shoots with blossom blight cankers were examined on each tree.

Table 2.8. Comparison of the ability of three Q_oI fungicides to inhibit sporulation of *Monilinia fructicola* on peach blossom blight cankers

Fungicide	Sporulation incidence[z] (% sporulating cankers)	Conidia production[z] (#conidia/canker)	Conidia production (adj)[z] (#conidia/mm canker)
Control	54.5 a	35678 a	2209 a
Azoxystrobin	44.8 ab	14032 b	970 b
Pyrac + bosc	41.2 b	16162 b	1035 b
Trifloxystrobin	37.6 b	9531 b	632 b

[z] Means in the same column followed by the same letter are not significantly different according to the Waller-Duncan *K*-ratio *t*-test (α=0.05, *K*=100). Each fungicide treatment mean was calculated from observations on cankers from 15 shoots/tree x 4 replicate trees/treatment x 2 experimental replications.

2.4 Discussion

The anti-sporulant activity of Q_oI fungicides against *Monilinia fructicola* fruit infections on peach was dependent on fungicide type and number of applications. Although all three fungicides examined were of the same Q_oI chemistry, trifloxystrobin was observed to be the most effective anti-sporulant, azoxystrobin had intermediate capabilities, and pyraclostrobin + boscalid was least active. At current labeled rates of application, trifloxystrobin, azoxystrobin, and pyraclostrobin + boscalid reduced sporulating area on average, across all treatments, by 42.4%, 15.9%, and 0.4%, respectively. Furthemore, two consecutive applications of Q_oI fungicides prior to inoculation also significantly reduced sporulating area of lesions in both years examined. Trifloxystrobin provided the greatest benefit with consecutive sprays, reducing sporulating area by 53-60%. These results demonstrate that trifloxystrobin, and to a lesser extent azoxystrobin, can provide curative, anti-sporulant activity against *M. fructicola* fruit infections in addition to preventative activity, as previously reported for many pathosystems (Margot *et al*., 1998).

A variety of studies have demonstrated the systemic activity of Q_oI fungicides. A very low but steady systemic uptake of trifloxystrobin was reported to occur during the subsequent 21-days following application to apple leaves, with the greatest increase occurring during the first seven days (Knauf-Beiter, 2000). A small, but biologically active amount of trifloxystrobin moved below the cuticle, providing curative activity against pathogens close to the surface (Margot *et al*., 1998). A similar effect was seen with azoxystrobin providing protective and curative activities against leaf spot and powdery mildew on sugar beet due to transliminar and systemic properties (Anesiadis *et*

al., 2003). Furthemore, root uptake of azoxystrobin has been demonstrated in pearl millet and similar systemic effects have been reported on other cereal crops (Sudisha *et al*., 2005). In our studies, the highest reductions in sporulating area at labeled rates were achieved by trifloxystrobin (44-89%) and azoxystrobin (14-66%) when inoculation occurred seven days after fungicide application. Although these results suggest that time was needed for absorption of the fungicides into the fruit tissues, these treatments were not significantly different from those in which inoculation occurred 24 hours after fungicide application. However, because it has a high lipophilicity (lg P_{ow} 4.5), trifloxystrobin is rapidly absorbed and retained by the waxy layers of the plant surface (Knauf-Beiter, 2000; Sauter *et al*., 1999). In contrast, azoxystrobin has a slightly lower lipophilicy (lg P_{ow} 2.5) but a slower metabolic breakdown, thereby allowing it to create a protective layer in the plant tissue (Sauter *et al*., 1999). Thus, regardless of the time period between fungicide application and inoculation, sufficient active ingredient was present to inhibit sporulation. That is, *M. fructicola* mycelium must move toward and through the fungicide-laden cuticle and epidermis in order to produce conidiophores and conidia. Due to the slow systemic movement of fungicide, this protected layer may have been somewhat deeper for the delayed-inoculation treatment, which may explain the consistent, but non-significant increase in anti-sporulant activity.

In general, the three Q_oI fungicides examined had less of an inhibitory effect on fruit colonization than on sporulation. When applied at maximum labeled rates in the various treatments, pyraclostrobin + boscalid, azoxystrobin, and trifloxystrobin reduced colony growth on average by 7.4%, 12.3%, and 7.5%, respectively. This difference in activity demonstrated the lack of deeper systemic movement of the fungicides into the

fruit tissue, as has been previously reported for trifloxystrobin (Margot *et al*., 1998). Thus, once injected into the fruit mesocarp below the pre- or post-treated epidermis, the pathogen was free to colonize tissues outward and downward, drawing upon the vaste stores of fruit nutrients. Had the fungicides quickly moved into the fruit interior, this colonization process may have been reduced. Indeed, pyraclostrobin has been shown to inhibit mycelial growth of *Cercospora beticola* on sugar beet and trifloxystrobin reduced *Uncinula necator* colony size on grape leaves (Karadimos *et al.*, 2005; Reuveni, 2000; Bartlett *et al.*, 2002). However, in these cases, the fungicides were in direct contact with the pathogen growing exposed on leaf surfaces. In contrast, on mature infected peach fruit, *M. fructicola* can quickly move into the fruit flesh, avoiding contact with any post-infection applied fungicides limited to the epidermis and cuticle.

Fungicides with curative properties can reduce sporulation of a fungal pathogen by decreasing the sporulating area of lesions and/or by reducing the density of spores produced on those lesions. Although our fruit infection studies focused on quantifying the reduction in sporulating area, conidia numbers were estimated in the separate blossom blight canker study. These latter results clearly showed significant reductions in spore production by all three Q_oI fungicides. As observed with reductions in sporulating areas on fruit, trifloxystrobin provided the greatest inhibition of conidia production on cankers, followed by azoxystrobin. However, unlike the fruit results, pyraclostrobin + boscalid also significantly reduced spore numbers on cankers. These results suggest that pyraclostrobin + boscalid may only act as an anti-sporulant by reducing spore density. Additional conidia production data are needed from fruit to confirm this hypothesis.

The rate of increase in rot lesion diameter for various pathogens on different crops, including peach, varies considerably at different stages of fruit maturity (Hall, 1972). In our studies, *M. fructicola* lesions on nontreated 'Autumnglo' fruit increased in diameter at rates ranging from 0.48 mm/h in 2006 to 0.95 mm/h in 2007. Similarly, nontreated lesions on 'Suncrest' fruit increased in diameter at rates ranging from 0.54 mm/h in 2006 to 0.94 mm/h in 2007. These rates of lesion expansion, estimated by averaging the minor and major ellipse axes, agree favorable with previously published rates of 0.59 – 0.92 mm/h for *M. fructicola* on peach (Hall, 1972). Although our calendar dates of inoculation were similar across years, fruit were much more visibly ripe in 2007, indicating a higher degree of susceptibility and therefore rate of lesion growth. Under these more susceptible conditions on 'Suncrest' fruit, trifloxystrobin continued to provide a significant reduction in sporulating area, whereas azoxystrobin failed to yield this benefit.

Brown rot resistant peach cultivars are not commercially available, and the presence of some overwintering inoculum can usually be assumed (Ogawa *et al*., 1995). Thus, the development of a severe epidemic in any given year is primarily dependent on the favorableness of weather conditions during the two month harvest season. In our studies, established lesions on fruit began sporulating at approximately 4 days after inoculation. Given this latent period and continuously favorable weather conditions for infection, then a minimum of 15 infection events are possible over the 60-day harvest period. Of course, since the infectious period for infected fruit is fairly long, many more concatenating infection cycles can occur. Under these conditions, deployment of fungicides that provide curative as well as protective activity, such as the Q_oIs, should

enhance management of the epidemic. Indeed, we propose that in disease favorable years, application of the Q_oIs at early stages of the epidemic is preferable to provide the greatest benefit in reducing the rate of disease increase. Similarly, if much blossom blight were observed or many mummies were present, then early application of a Q_oI would reduce inoculum from these sources, although additional data are needed to confirm anti-sporulant capabilities on mummified fruit.

The results of this study suggest it may be adventageous to use Q_oI fungicides in a standard spray program for brown rot. Their ability to reduce sporulation combined with the protective activities of the widely used DMI fungicides should provide excellent control of the disease. Furthermore, given the emergence of resistance in *M. fructicola* to the DMI fungicides (Luo *et al*., 2008), the Q_oIs can play an important role in delaying onset of resistance to the DMIs. Similarly, the DMIs can aid in preventing development of resistance to the Q_oIs. However, some recent results showed that DMI-resistant strains of *M. fructicola* are not controlled by Q_oI fungicides (Luo and Schnabel, 2008). Thus, an integrated Q_oI and DMI brown rot management program may only be viable for orchards that initially contain pathogen strains sensitive to both fungicide chemistries.

3. FIRST REPORT OF THE PEACH BROWN ROT FUNGUS *Monilinia fructicola* RESISTANT TO DEMETHYLATION INHIBITOR FUNGICIDES IN NEW JERSEY.

Reduced sensitivity and resistance of *M. fructicola* to demethylation inhibitors (DMIs; fungicide group 3) have been previously found in stone fruit orchards in Georgia, South Carolina, Ohio, and New York (Luo *et al*., 2008). Such resistance development is of major concern given the importance of DMIs for brown rot management. Eleven single-spore isolates, originally collected in 2006 from separate commercial peach (*Prunus persica*) orchards in southern New Jersey, were removed from cold storage in early 2008 and examined *in vitro* for resistance to the DMI propiconazole (Orbit 3.6EC; Syngenta Crop Protection, Inc., Greensboro, NC). After 19 months cold storage, isolate 007 was inhibited 53.4% in growth on PDA amended at the discretionary dose of 0.3 µg/ml propiconazole; inhibition of the remaining isolates ranged from 81.4% to 100%. Inhibition values were based on two replications of eight colony observations per isolate performed after 4-days incubation at 25°C. Given the previously reported relationship between duration of cold storage and propiconazole sensitivity, isolate 007 was tentatively deemed resistant (Cox *et al*., 2007). To confirm the *in vitro* results, isolates were grown at 25°C on cellophane covered PDA for 7 days. Genomic DNA was isolated from mycelium using the DNeasy Plant Mini Kit (Qiagen, Inc., Valencia, CA). A PCR was run on a GeneAmp thermal cycler (Applied Biosystems, Inc., Foster City, CA) using a previously described program and primers INS65-F and INS65-R that flank a 65-bp region named 'Mona' specific to DMI resistant isolates (Luo *et al*., 2008). PCR products were separated via electrophoresis on 0.8% agarose gel. The primers amplified a 376-bp

fragment from isolate 007 and a 311-bp fragment from all other isolates, thus indicating the presence of Mona in 007. A restriction fragment length polymorphism (RFLP) analysis using the BsrBI enzyme, specific to a single restriction site identified within Mona, was conducted on the amplified fragments from all isolates. Electrophoresis results showed digestion of the 376-bp fragment from 007 into 140-bp and 236-bp fragments, thereby confirming presence of Mona; none of the 311-bp fragments from the remaining isolates were digested by the BsrBI enzyme. Although economic loss from brown rot has not been reported in New Jersey, these results show that propiconazole-resistant *M. fructicola* currently exists in commercial peach orchards in the state. Additional, more extensive sampling is planned to ascertain the prevalence and location of resistant strains. To combat this risk, brown rot control programs are incorporating quinone outside inhibitors (Q_oIs; fungicide group 11) and carboxamides (fungicide group 7) into control programs as a resistance management strategy. However, these fungicides are also at risk of resistance development. Furthermore, a strong correlation between DMI resistance and an accelerated resistance to Q_oIs in *M. fructicola* has been identified (Luo and Schnabel, 2008a). Thus, additional fungicide chemistries may be needed to avoid significant crop loss from brown rot in the future.

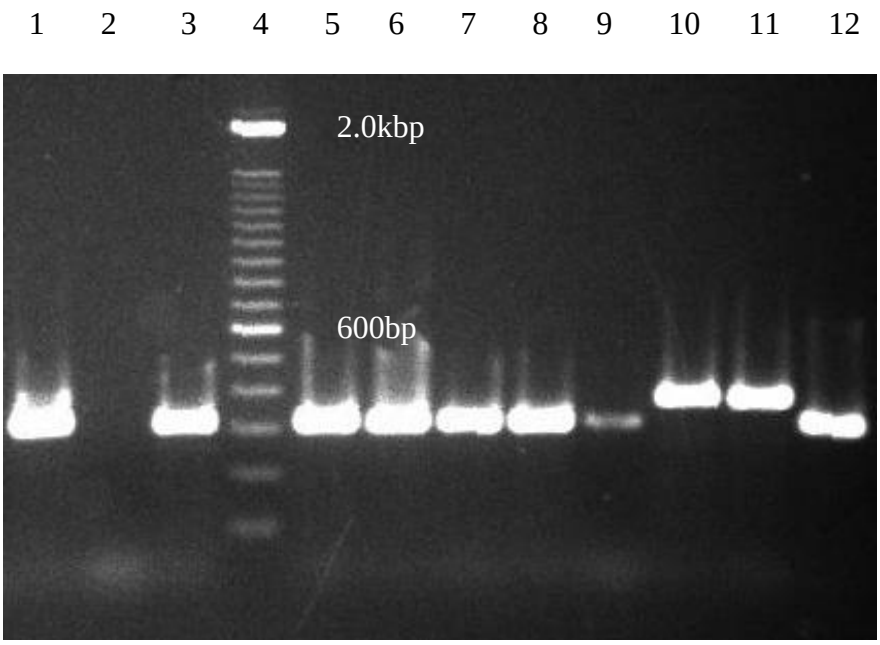

Figure 3.1. Gel electrophoresis analysis of polymerase chain reactions (PCR) of *Monilinia fructicola* isolates to detect the Mona element located upstream of the *MfCYP51* gene. Lane 4, 100 bp DNA Ladder (Invitrogen); lanes 1 to 3, isolates 016, 015 and 014 respectively; lanes 5 to 12, isolates 013, 011, 010, 009, 008, 007, 007, 006 respectively. Isolate 012 was not examined in this analysis.

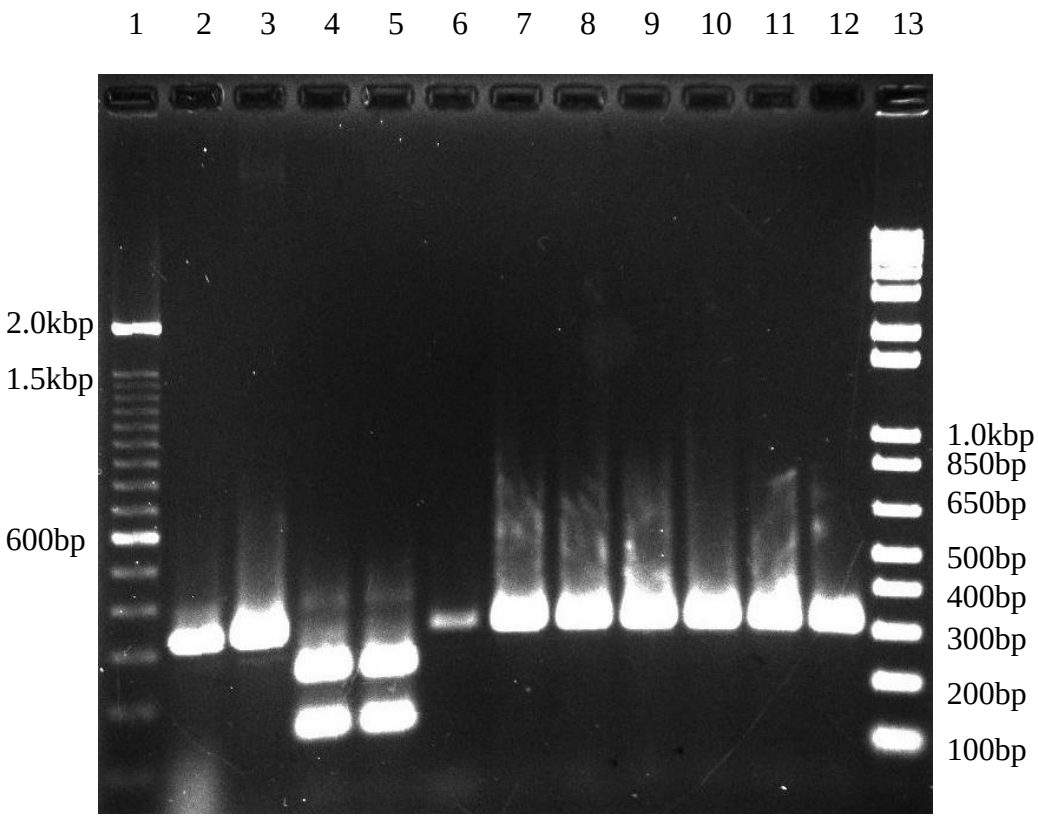

Figure 3.2. Gel electrophoresis analysis of endonuclease *Bsr*BI digested PCR fragments of *Monilinia fructicola* isolates with primers *INS65-F* and *INS65-R.* Lanes 1and 13, 100 bp DNA Ladder (Invitrogen) and 1 kb Plus DNA Ladder (Invitrogen), respectively. ; lane 2, isolate 006 uncut control (not exposed to enzyme); lane 3, isolate 006 uncut by enzyme; lanes 4 and 5, *M. fructicola* isolate 007 digested by *Bsr*BI; lanes 8 to 12, isolates 008, 009, 010, 011, 013, 014 and 016 respectively uncut by *Bsr*BI enzyme. Isolates 012 and 015 were not included in this analysis.

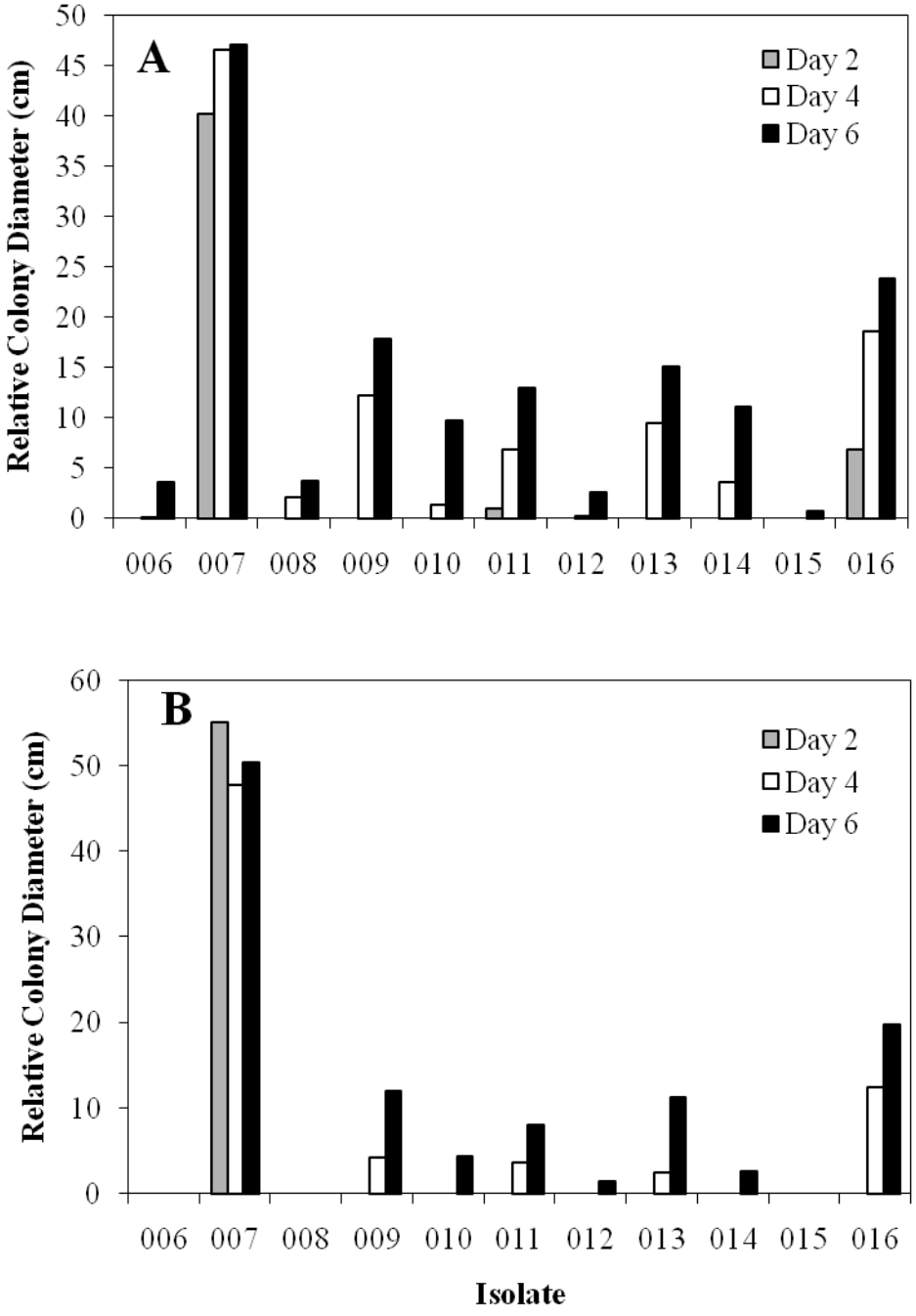

Figure 3.3. Comparison of relative colony diameter of 11 different isolates of *M. fructicola* after 2, 4, and 6 days incubation on PDA amended with 0.3 ppm propiconazole from (A) Orbit 3.6EC (B) Propimax 3.6EC fungicides.

4. REFERENCES

Agrios, G.N. 2005. Plant Pathology, Fifth Edition. Academic Press, NY.

Alexopoulos, C.J., Mims, C.W., and Blackwell, M. 2006. Introductory mycology, Fourth Edition. John Wiley and Sons, Inc., New York.

Anderson, H.W. 1956. Diseases of fruit crops. McGraw-Hill, NY. 189-206.

Anesiadis, T., Karaoglanidis, G.S., and Tzavella-Klonari, K., 2003. Protective, curative and eradicant activity of the strobilurin fungicide azoxystrobin against *Cercospora beticola* and *Erysiphe betae*. J. Phytopathology 151:647-651.

Bartlett, D.W., Clough, J.M., Godwin, J.R., Hall, A.A., Hamer, M., and Parr-Dobrzanski, B. 2002. Review: The strobilurin fungicides. Pest Management Science 58: 649-662.

Biggs, A.R. and Northover, J. 1988. Early and late-season susceptibility of peach fruits to *Monilinia fructicola*. Plant Disease 72:1070-1074.

Burnett, A.L. and Lalancette, N. 2007. Suppressive anti-sporulant activity of QoI fungicides on *Monilinia fructicola* on peach. Phytopathology 97:S15.

Burnett, A.L. and Lalancette, N. 2007. Effect of QoI fungicide rate and time of inoculation on brown rot lesion development on peach fruit. Phytopathology 97:S177.

Burnett, A. L. and Lalancette, N. 2006. Effect of QoI fungicides on colonization and sporulation by *Monilinia fructicola* on peach fruit. Phytopathology 96:S17.

Byrde, R.J.W and Willets, H.J. 1977. The brown rot fungi of fruit: their biology and control. Pergamon Press, New York.

Corbin, J.B. 1962. Factors determining the length of the incubation period of *Monilinia fructicola* (Wint.) Honey in fruits of *Prunus* spp. Australian Journal of Agricultural Research 14(1):51-60.

Cox, K.D., Bryson, P.K., and Schnabel, G. 2007. Instability of propiconazole resistance and fitness in *Monilina fructicola*. Phytopathology 97:448-453.

Emery, K.M., Michailides, T.J. and Scherm, H. 2000. Incidence of latent infection of immature peach fruit by *Monilinia fructicola* and relationship to brown rot in Georgia. Plant Disease 84:854-857.

Ferreira, E.M., Alfenas, A.C., Maffia, L.A., Mafia, R.G., and Mounteer, A.H. 2008. Effectiveness of systemic fungicides in the control of *Quambalaria eucalypti* and their effects on production of eucalypt mini-cuttings for rooting. Crop Protection 27:161-170.

Hall, R. 1972. Pathogenicity of Monilinia fructicola: Part III. Factors influencing lesion expansion. J. Phytopathology 73: 27–38.

Hamilton, G.C., Heckman, J.R., Lalancette, N., Majek, B.A., Paulin, J.B., Polk, D., and Ward, D.L. 2009. The New Jersey Commercial Tree Fruit Production Guide, New Jersey Agricultural Experiment Station, Rutgers Cooperative Extension, bulletin E002, 237 pages.

Holb, I. J. and G. Schnabel 2007. Differential effect of triazoles on mycelial growth and disease measurements of *Monilinia fructicola* isolates with reduced sensitivity to DMI fungicides. Crop Protection 26:753-759.

Hong, C.X., Holtz, B.A., Morgan, D.P., and Michailides, T.J. 1997. Significance of thinned fruit as a source of the secondary inoculum of *Monilinia fructicola* in California nectarine orchards. Plant Disease 81:519-524.

Joshua, Troy. 2006. New Jersey agriculture annual report: agricultural statistics. USDA's National Agriculatural Statistics Service, 97pp.

Karadimos, D.A., Karaoglanidis, G.S., and Tzavella-Klonari, K. 2005. Biological activity and physical modes of action of the Qo inhibitor fungicides trifloxystrobin and pyraclostrobin against *Cercospora beticola*. Crop Protection 24: 23-29.

Knauf-Beiter, G., Fischer, W., Laird, D., and Genay, J-P. 2000. The mesosystemic activity of trifloxystrobin. AFPP – Sixth International Conference on Plant Diseases, Tours, France, December 6-8, 2000.

Lalancette, N., McFarland, K.A., and Burnett, A.L. 2009. Inhibition of *Monilinia fructicola* sporulation on peach blossom blight cankers by QoI fungicides. Phytopathology 99: *in press*.

Lalancette, N., McFarland, K.A., and Burnett, A.L. 2008. Reduction in primary inoculum of *Fusicladosporium carpophilum* on stone fruit by the QoI fungicide trifloxystrobin. Phytopathology 98:S207.

Margot, P., Huggenberger, F., Amrein, J., and Weiss, B. 1998. CGA 279202: A new broad-spectrum strobilurin fungicide. Brighton Crop Prot. Conf. Pests Dis. 2:375-382.

Lee, M.-H., and Bostock, R.M. 2006. Induction, regulation, and role in pathogenesis of appressoria in *Monilinia fructicola*. Phytopathology 96: 1072-1080.

Luo, C.-X., Cox, K.D., Amiri, A., and Schnabel, G. 2008. Occurrence and detection of the DMI resistance-associated genetic element 'Mona' in *Monilinia fructicola*. Plant Disease 92: 1099-1103.

Luo, C.-X., and Schnabel, G. 2008a. Adaptation to fungicides in *Monilinia*

fructicola isolates with different fungicide resistance phenotypes. Phytopathology 98:230-238.

Luo, C.-X., and Schnabel, G. 2008b. The cytochrome P450 lanosterol 14- demethylase gene is a demethylation inhibitor fungicide resistance determinant in *Monilinia fructicola* field isolates from Georgia. Applied Environmental Microbiology 74:359-366.

Ogawa, J.M., Zehr, E. I., and Biggs, A. R. 1995. Brown rot. Pages 7-10 in: Compendium of Stone Fruit Diseases, J. M. Ogawa, E. I. Zehr, G. W. Bird, D. F. Ritchie, K. Uriu, and J. K. Uyemoto, eds., The American Phytopathological Society, St. Paul, MN.

Phillips, D.J. 1984. Effect of temperature on *Monilinia fructicola* conidia produced on fresh stone fruits. Plant Disease 68:610-612.

Reuveni, M. 2001. Activity of trifloxystrobin against powdery mildew and downy mildew diseases of grapevines. Canadian Journal of Plant Pathology 23:52-59.

Rohel, E.A., Cavelier, N., and Hollomon, D.W. 2001. Microscopic analysis of the effect of azoxystrobin treatments on *Mycosphaerella graminicola* infection using green fluorescent protein (GFP)-expressing transformants. Pest Management Science 57:1017-1022.

Sauter, H., Steglich, W., and Anke, T. 1999. Strobilurins: evolution of a new class of active substances. Agnew. Chem. Int. 38:1328 -1349.

Schnabel, G., Bryson, P. K., Bridges, W. C., and Brannen, P. M. 2004. Reduced sensitivity in *Monilinia fructicola* to propiconazole in Georgia and implications for disease management. Plant Disease 88:1000-1004.

Sudisha, J., Amruthesh, K.N., Deepak, S.A., Shetty, N.P., Sarosh, B.R., and Shetty, H.S. 2005. Comparative efficacy of strobilurin fungicides against downy mildew disease of pearl millet. Pesticide Biochemistry and Physiology 81:188-197.

Veloukas, T., Bardas, G.A., Karaoglanidis, G.S., and Tzavella-Klonari, K. 2007. Management of tomato leaf mould caused by *Cladosporium fulvum* with trifloxystrobin. Crop Protection 26:845-851.

Watson, W.A., Zehr, E.I., and Grimes, L.W. 2002. Influence of temperature and wetting period on inoculum production by Monilinia fructicola in peach twig cankers. Plant Disease 86:666-668.

Weaver, L.O. 1950. Effect of tempaerature and relative humidity on occurrence of blossom blight of stone fruits. Phytopathology 40: 1136-1153.

Whetzel, H.H. 1945. A synopsis of the genera and species of the Sclerotiniaceae, a family of stromatic inoperculate discomycetes. Mycologia 37:648-714.

Wong, F.P., and Wilcox, W.F. 2001. Comparative physical modes of action of azoxystrobin, mancozeb, and metalaxyl against *Plasmopara viticola* (grapevine downy mildew). Plant Disease 85: 649-656.

Zehr, E.I. 1982. Control of brown rot in peach orchards. Plant Disease 66:1101-1105.

5. APPENDIX

5.1 Fruit Inoculation Technique

Brown rot occurs naturally in the field as peach fruit mature at the end of a season. This natural infection varies depending on environmental conditions and peach variety. A peach variety with a low susceptibility to brown rot combined with a very cool and dry time period at harvest will yield very little disease pressure. To effectively experiment with fungicides in the field, artificially inoculating with *M. fructicola* guarantees an infection and a disease level to quantify. Artificially inoculating fruit is not the same as conditions a grower would encounter; however it is necessary to have infection in the experiment to investigate curative fungicidal activity. The purpose of this experiment was to develop a method of inoculation that is manageable and quantifiable.

One method of artificial inoculation considered was applying a conidial suspension with an atomizer directly on a maturing peach fruit until completely covered. The fruit was bagged for 24 hours to provide a controlled area of high humidity. This method was ruled out, despite its resemblance to naturally occurring field conditions, due to the possibility of multiple infection sites appearing on the fruit. Multiple lesions on one fruit would quickly accelerate the disease so to complete destruction of the fruit within three days. Using immature instead of mature peaches might slow down the lesion development, but that method also has problems. Immature peaches are very resistant to brown rot because of high levels of phenolic compounds found in the epidermal cells (Agrios, 2005). These compounds, such as caffeic acid, inhibit cutinase activity found in the tips of germ tubes and appressoria of *M. fructicola* (Agrios, 2005). This is why immature peach fruit are not nearly as susceptible to brown rot infection as

maturing fruit. It is also why spraying inoculum on an immature peach would be an ineffective means of infection development.

The most effective method of attaining quantifiable disease was to inject early maturing fruit using a syringe. This method proved to be the most efficient because it ensured disease development without being overly labor intensive and expensive. In this experiment we set out to determine an optimal concentration and volume of spores to successfully produce sporulating lesions on maturing fruit. Another objective was to create a lesion that developed slow enough to measure its rate of expansion over 7 days. Our hypothesis stated that the highest concentration of spores delivered at the highest volume would develop the bigger lesions and lesser concentrations and volumes of each would yield a lesser lesion. We also thought that even at the highest volume and concentration, the lesion would still develop slowly enough to allow multiple ratings over a seven day period.

Ten ripening peach fruit were collected from 'Autumnglo' trees per treatment. We tested four different concentrations measured in the number of conidia/ml; 10, 100, 1000 and 10000. The different volumes tested were: 0 ml (just a wound created by the syringe holding a spore suspension of the desired concentration), 0.05 ml and 0.1ml delivered by the syringe. Evaluations were made 5 days after inoculation. There were visible differences in sizes of lesions therefore a rating scale of 0 to 3 was used with 0=no disease and 3=largest lesion development.

The results show the most disease occurred in with the 10,000 conidia/ml applied at 0.1ml and 0.05ml (Table 5.1). Interestingly the fruit that receive just a wound from the syringe still developed some lesions at the higher concentration, however just to be sure

infection occurred we use 0.1ml of a 10,000 conidia/ml for all experiments. From these results we determined that we could successfully measure the growth of the lesions and development of sporulation over the course of a week.

Table 5.1. Ratings of the inoculation technique experiment using 'Autumnglo' peach fruit. These ratings are the average visual ratings of ten peaches inoculated for each treatment. The rating scale ranged from 0 to 3 with 0=no disease, 1=small lesion, 2=medium lesion, 3=large lesion.

Concentration (conidia/ml)	Voume Injected (ml)		
	0.1	0.05	0
10,000	3	3	1.3
1,000	2.6	2.6	0.1
100	1.7	1.4	0.7
10	0.7	0.5	0.1

5.2 Fungicide Dip Study

In order to plan an efficient field trial, it is important to determine what chemicals should be included to match the desired outcome. Given unlimited resources and time, comparing all these fungicides would be the best option, however, the field trial needed to be limited. Field trials in 2006 were aimed at determining how Q_oI fungicides affected brown rot lesion and sporulation development, so in 2005 we set up a small scale experiment. The study involved six fungicides, 3 strobilurins and 3 DMIs, to determine which would best control brown rot, caused by *M. fructicola*. DMI fungicides are currently used to prevent brown rot, so their involvement in this assay was to act as a positive control (the negative control being untreated fruit).

The fungicides examined in this experiment are similar to previously mentioned in Chapter 2. They were: azoxystrobin at 280.2 g/ha and 560.4 g/ha and propiconazole at 302 g/ha and 604 g/ha(Abound 2.08F and Orbit respectively; Syngenta Crop Protection, Greensboro, NC); trifloxystrobin at 140.1 g/ha and 280.2 g/ha and tebuconazole at 280 g/ha and 561 g/ha(Flint 50WG and Elite 45DF respectively; Bayer CropScience, Research Triangle Park, NC); and pyraclostrobin + boscalid at 130.0 + 256.8 g/ha and 260.0 + 513.6, respectively (Pristine 38WG; BASF Corp., Research Triangle Park, NC); fenbuconazole at 140g/ha and 280g/ha (Indar 75WSP; Dow AgroSciences, Indianapolis, IN). These rates are the standard rates (1X) and double the standard rates (2X) for control of brown rot. Approximately 500 ml of each fungicide was prepared in a jar in which a peach would be dipped into and held for 10 seconds, then placed in a tray for future inoculation. Ten peaches at the fruit ripening stage were used for each treatment. After 24 hours a syringe was used to inject each fruit with 0.1ml

of a 10,000 conidia/ml suspension. Measurements of the lesion areas and sporulating areas were taken 3 and 5 days post inoculation.

The results indicated that Orbit was able to provide absolute protection against the pathogen at a 2X rate (Table 5.2). Orbit was the also the best treatment at both rates to control disease development again at the 2X rate. Flint was the best treatment to control sporulation at the 2X rate out of all the treatments. Of the strobilurins, Pristine was the most effective at controlling lesion development at both rates. These results led us to include all three strobilurin treatments and Orbit into our later experiments. We ultimately decided not to use the Dip method as our experimental design. This study worked with detached fruit and we thought it important for the fruit to be in as typical a field setting as possible. Bringing fruit in to be treated and inoculated presented unknowns that would affect outcomes of how the chemical would be applied and may affect the chemicals movement through the host system.

Table 5.2. Mean lesion and sporulating areas of ‘Autumnglo’ fruit in the fungicide dip study. Ten fruit were used per treatment.

Treatment	Rate	Lesion Area (cm2)	Sporulating Area (cm^2)
Control	-	44.9	11.9
Abound	1X	36.2	6.3
	2X	29.9	5.4
Flint	1X	39.0	2.1
	2X	38.4	0.6
Pristine	1X	30.3	4.8
	2X	24.8	4.7
Elite	1X	23.2	6.2
	2X	18.1	4.7
Indar	1X	32.1	9.4
	2X	31.0	8.6
Orbit	1X	14.0	1.0
	2X	0.0	0.0

5.3 Relationship between QoI Concentration and *In Vitro* Growth of *Monilinia fructicola*.

5.3.1 Introduction

One of the most practical means of examining how well a fungicide prevents disease is to perform a field trial. The information gathered from a field trial can easily translate into what a grower can do to create the highest crop yield for the season. Field trials can only occur when the fungicide is needed and usually can only be performed once a season, especially in the case of controlling brown rot on peaches. Another means of learning about the potential a fungicide has in acting against a fungus is to set up an *in vitro* study.

We wanted to know how the strobilurin fungicides control fungal growth in optimal growth conditions for *M. fructicola in vitro*. Since the pathogen produces copious amounts of mycelia and spores *in vitro*, we decided to add the fungicides to the media to see the effects. This *in vitro* study included trifloxystrobin, azoxystrobin and pyraclostrobin + boscalid at sublethal and lethal doses. In the first part of this experiment Mf007, a DMI-resistant isolate, was examined. However after reviewing the preliminary results from this study, we decided that DMI-sensitive isolates should also be studied in case this trait influenced activity of the strobilurin fungicides. Isolates Mf006, Mf007 and Mf015 were chosen based on their ability to colonize and sporulate well on PDA.

The main goals of this experiment were to estimate ED_{50} values for control of colonization and sporulation, compare the effectiveness of each fungicide, and determine how to use strobilurin fungicides at their greatest efficiency. Based on our field studies,

we hypothesized that trifloxystrobin will control sporulation better than azoxystrobin and pyraclostrobin + boscalid, but would not be able to control mycelial growth as well.

5.3.2 Materials and Methods

Experimental Design. The 1X concentrations of the fungicides examined in the experiments were azoxystrobin at 0.150 g/L (Abound 2.08F; Syngenta Crop Protection, Greensboro, NC); trifloxystrobin at 0.074 g/L (GEM 500SC; Bayer CropScience, Research Triangle Park, NC); and pyraclostrobin at 0.069 g/L (Cabrio; BASF Corporation, Research Triangle Park, NC). The entire experiment was repeated once.

Media. To get optimal growth, *M. fructicola* was grown on Potato-dextrose agar (PDA) made as directed with 39.0g PDA mix per 1.0L tap water. After the mixture was autoclaved at 250°C and cooled back down to 55°C, SHAM (salicylhydroxamic acid) was added at 100mg/ug/ml. Fungicides were added at 10-fold dilutions from 1X to 0.00001X and mixed thoroughly. Each Petri dish received 20ml of the amended solution.

Inoculum. Single-spore isolates Mf006, Mf007 and Mf015, which were found in three different Southern New Jersey orchards, were grown on V8 agar for seven days. A cork borer with a diameter of 3.0mm was used to transfer plugs from each isolate on V8 to the fungicide amended PDA.

Assessment. Seven days post inoculation the long and short diameters of the fungal colonies were measured. Areas for these colonies were found using the formula for an ellipse. Conidia were then harvested by washing off the colonies with deionized water using a DeVilbiss atomizer set at 34.5 kPa (DeVilbiss Health Care, Somerset, PA). Collected spores were counted using a hemacytometer.

5.3.3 Results

The relative colony diameters of isolates Mf006 and Mf007suggest a strong rate response of all three fungicides (Fig. 5.1A,B) The ED_{50} values for relative colony diameter indicated azoxystrobin required the highest concentration to control colony development. Trifloxystrobin required the lowest concentration which put the efficiency of pyraclostrobin controlling colony development in the middle. Data from both isolates agree that trifloxystrobin is better at controlling colony development than both pyraclostrobin and azoxystrobin. Complications with isolate Mf015 prevented any analysis.

The relative spore density ED50 values were the lowest for both isolates growing on trifloxystrobin, indicating that this fungicide was the most effective anti-sporulant. (Fig. 5.2A, B). Similarly, pyraclostrobin proved to be slightly better than azoxystrobin at inhibiting sporulation (Fig 5.2A, B). It is worth noting, when each fungicide was examined at the lowest concentration, both isolates produced more spores per colony area than the controls. This may suggest hormesis, or a stimulation of sporulation, is occurring; however further studies are needed to validate this phenomenon.

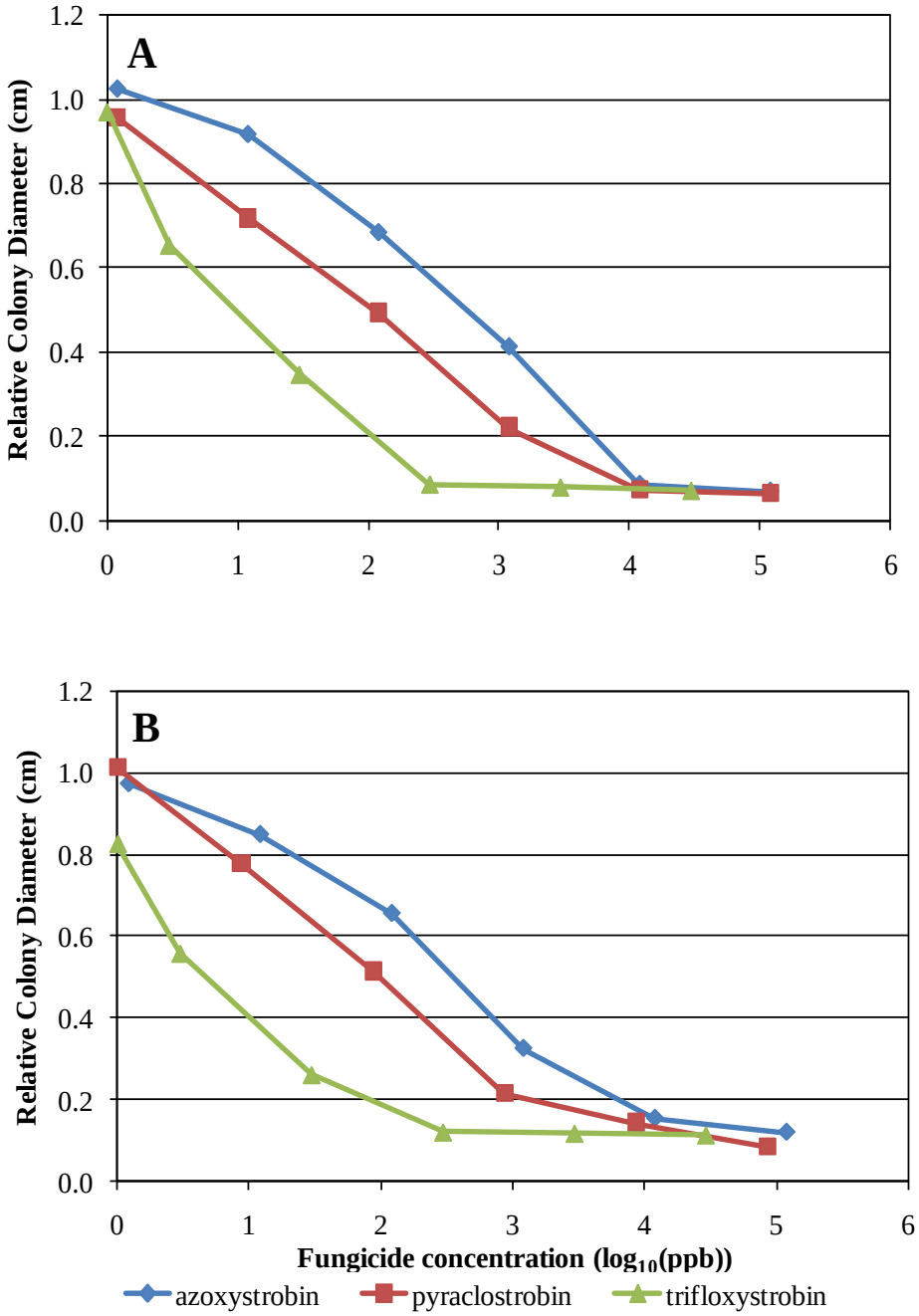

Figure 5.1. The effect of fungicide concentration on the colony diameter relative to the control for isolates Mf006 (A) and Mf 007 (B) grown on fungicide amended PDA + SHAM.

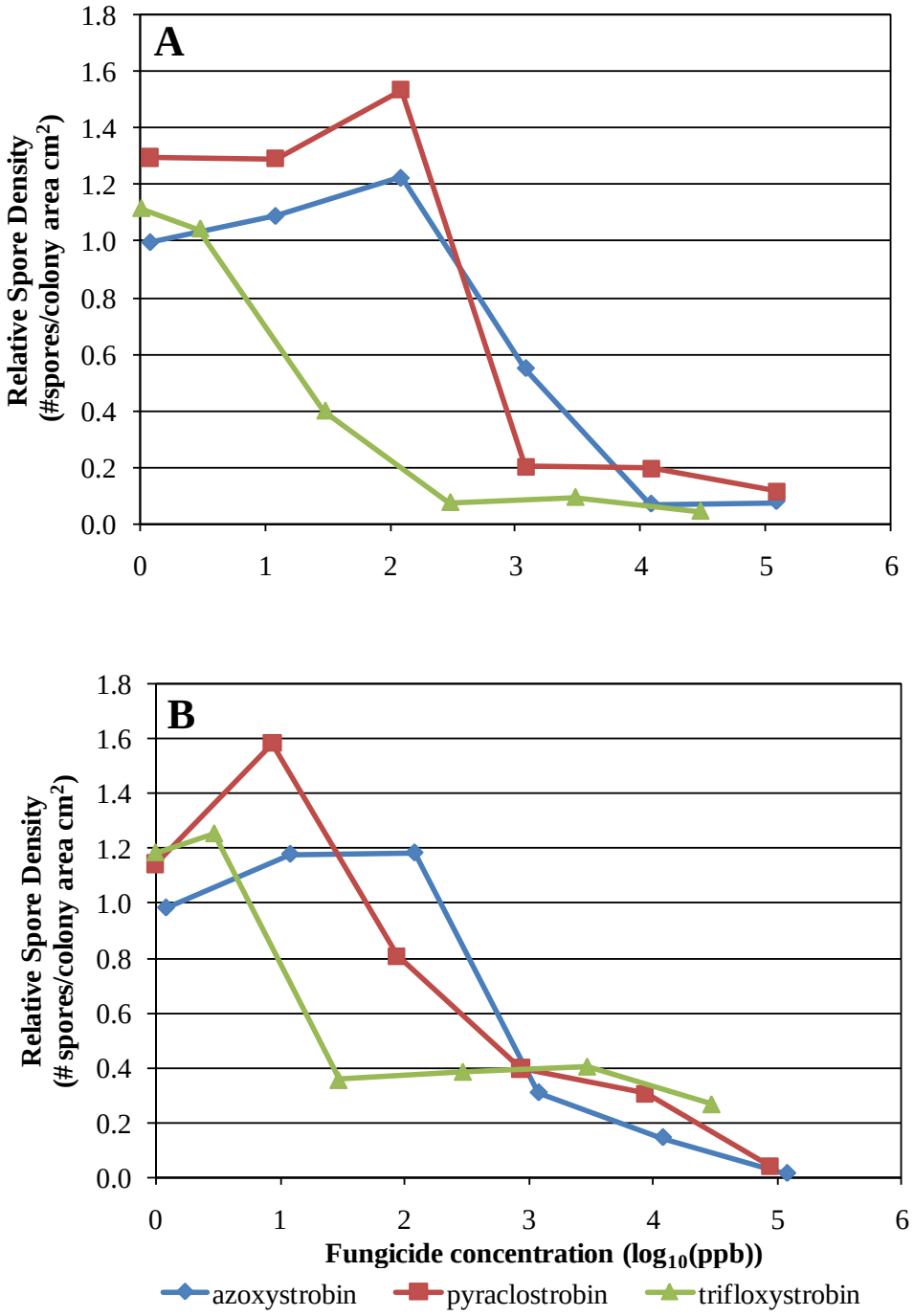

Figure 5.2. The effect of fungicide concentration on the spore density relative to the control for isolates Mf006 (A) and Mf007 (B) grown on fungicide amended PDA+SHAM.